# *Rhizosphere bacteria are more strongly related to plant root traits than fungi in temperate montane forests: insights from closed and open forest patches along an elevational gradient*

# Rhizosphere bacteria are more strongly related to plant root traits than fungi in temperate montane forests: insights from closed and open forest patches along an elevational gradient

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**Summary**

**Aims:**

Heterogeneous canopies in temperate montane forests affect microclimate and soil characteristics, with important effects on soil microbial communities and related processes. Here, we studied the interactions between plant root traits and soil bacterial and fungal communities in closed forest and gaps in a mixed forest along an elevational gradient in the French Alps (1400, 1700 and 2000 m).

**Methods:**

Samples were separated into three fractions (plant root endosphere, rhizosphere and bulk soil), to further investigate the influence of plant zones on microbial communities. Bacterial (16S) and fungal (ITS) biodiversity was determined using high throughput sequencing, along with standard measures of soil, litter and root traits.

**Results:**

We found that (i) microbial community diversity was higher in gaps than in closed forest because of increased root trait diversity and density; (ii) open versus closed forest patches affected phylogenetic dispersion despite differences in elevations with phylogenetic clustering in closed forest; (iii) the interaction between root traits and microbial communities was stronger for rhizosphere and endosphere compartments than for bulk soil and (iv) bacterial community composition was better explained by root traits than for fungi.

**Conclusions:**

Our findings highlight the importance of open gaps versus closed forest patches and associated root traits affecting microbial community structure, particularly for bacterial assemblages that exhibited a stronger interaction with root traits than for fungi.

**Key words:** Bacteria; closed forest; fungi; gaps; ITS; phylogenetic clustering; root traits; soil properties; 16S.

## Introduction

Naturally heterogeneous canopies in forests create a mosaic of belowground responses in the activity and composition of microbial communities, that are linked primarily to the presence of specific plant species, as well as heterogeneity in microclimate and soil characteristics (Lladó et al. 2018; Muscolo et al. 2014). Disturbance in closed forests causes gaps that can alter local soil moisture, solar radiation and air and soil temperatures (de Freitas and Enright 1995; Gray et al. 2002). Understorey species then dominate in the gap, until tree growth causes the canopy to close again. Recent evidence shows that gap size alters soil microbial community structure (Muscolo et al. 2014; Yang et al. 2017a; Yang et al. 2017b) but it is not clear if these changes were primarily due to differences in the composition of plant species and/or to modifications in the local microclimate. Individual trees also structure soil microbial communities (Bach et al. 2010) through: (i) a modification in soil physicochemical parameters mostly via changes in litter quantity and quality (Baldrian 2017; Dukunde et al. 2019; Prescott and Grayston 2013; Saetre and Bååth 2000), (ii) root traits and root exudates in the rhizosphere (Colin et al. 2017; Lladó et al. 2018), in addition to (iii) the modifications in microclimatic conditions mentioned above. Here, we aim to identify whether gaps in naturally heterogeneous forests affect bacterial and fungal community structure through changes in microclimate, soil physicochemical parameters or vegetation, with a specific focus on root traits of both trees and understory species. To achieve our objective, we use an elevational gradient to observe if shifts in soil microbial diversity between gaps and closed forests are reflected in diverse climatic and soil conditions (but with minimal differences in soil texture).

Studying forest microbial diversity and structure along climate and soil gradients is challenging because factors can co-vary (McCain and Grytnes 2010). Nevertheless, elevational gradients permit the study of several abiotic factors, since major changes in these factors are found along relatively short distances (Körner 2007; Ren et al. 2018). Based on bacterial taxonomic diversity, contradicting results have been found in temperate regions, with taxonomic richness varying along elevational gradients; either decreasing (Bryant et al. 2008), showing a hump-backed

relationship (Singh et al. 2012), or not showing any relationship (Shen et al. 2014). Fungal richness has been observed to either decrease at high elevation (Bahram et al. 2012; Kernaghan and Harper 2001), or have a hump-backed shape along the gradient (Miyamoto et al. 2014). Several studies have included both fungal and bacterial communities, but trends varied (Ren et al. 2018; Shen et al. 2014; Siles and Margesin 2016). These contradicting results are possibly because different environmental variables along the elevational gradient affect community assemblages (Coince et al. 2014). Here, our approach is to examine microbial diversity in gaps and closed forest along an elevational gradient, to determine if the patterns observed in response to local differences are repeated along the elevational gradient.

Any modifications in soil microbial community assemblages will have a cascade of effects on soil structure and nutrient cycling, including: (i) soil aggregation and aggregate stability (Baumert et al. 2018; Chenu and Sotzky 2002) and (ii) decomposition processes (Kohout et al. 2018; Schneider et al. 2012). Gaps in forests might be beneficial to microbial communities through increases in their biomass and activity (Muscolo et al. (2014); Yang et al. (2017b)). If microorganisms are more abundant and active in gaps, soil aggregate stability and litter decomposition should be enhanced. However, these processes are also linked to the plant species present and their chemical and physiological traits (Grigulis et al. 2013; Poirier et al. 2018). Prescott and Grayston (2013) found that the main factors associated to differences in microbial communities in litter, forest floors and soil were pH and base cation content of the litter and whether the trees were broadleaf or coniferous. Besides, Brant et al. (2006) revealed that in forest ecosystems of Oregon, Pennsylvania, and Hungary, root carbon (C) inputs exerted a larger control on microbial community composition than litter inputs. Plant species diversity and root density are also greater in gaps compared to closed forests (Mao et al. 2015) due to a higher presence of shrubs, forbs and grasses, benefitting the activity of rhizosphere microbial communities (Kuzyakov and Blagodatskaya 2015). Therefore, even though a higher input of litter is expected in closed forests, the increase in root density and diversity, and associated litter, could result in more diverse and active microbial communities in gaps.

Approaches for studying microbial diversity along gradients have changed from taxonomic to phylogenetic in recent years (Parks and Beiko 2013). Phylogenetic clustering processes are observed when evolutionarily related organisms coexist due to restricting abiotic or biotic conditions. Several studies have found that phylogenetic clustering increases with elevation for bacterial communities (Bryant et al. 2008; Wang et al. 2012; Zhang et al. 2018). However, this filtering (i.e. environmental selection against certain species) is not only mediated by abiotic factors along the gradient but also by biotic interactions (Goberna et al. 2014a; Mayfield and Levine 2010) and so, there might be a role of gaps and closed forests in phylogenetic patterns. To the best of our knowledge, there is no study of the effects of gaps and closed forests in temperate forests, where changes in root diversity and density and microclimatic conditions are expected to influence strongly the bacterial phylogenetic patterns.

Ecological habitat (i.e. rhizosphere or bulk soil) is the main factor structuring bacterial communities (Uroz et al. 2010), due primarily to the supply of rhizodeposits released from live roots (Philippot et al. 2013; Shi et al. 2012). Rhizodeposits include root cells and tissues, exudates, mucilage, volatiles and soluble lysates that are sloughed-off as a root grows through soil (Uren 2000). However, recent studies have shown that the structure of fungal communities are not related to these rhizodeposits, but are strongly related to tree species (Urbanová et al. 2015; Uroz et al. 2016) mainly through litter quality that affects community composition of saprotrophic and ectomycorrhizal (ECM) fungi (Aponte et al. 2013; Prescott and Grayston 2013). If root traits differ between gaps and closed forest, modifications in bacterial communities in particular, should be observed within the rhizosphere compared to bulk soil.

Using an elevational gradient to observe if patterns of microbial diversity and structure between gaps and closed forest are repeated along the gradient, we aim at addressing four hypotheses. First, we hypothesize that microbial community diversity will be higher in gaps than in closed forest because of increased root trait diversity and density. Second, we expect to observe phylogenetic clustering in closed forest due to reduced root trait diversity and density compared to gaps. Third, we hypothesize that the interaction between root traits and microbial communities

will be stronger for rhizosphere and endosphere compartments than for microbial communities inhabiting bulk soil. Finally, we expect that bacterial communities will be more strongly affected by ecological habitat (bulk soil, rhizosphere or endosphere) than fungal communities. These modifications to patterns in community assemblage should be repeated along the elevational gradient, because plant communities should have a greater effect on structuring microbial communities than abiotic factors, because of the habitat quality that they provide.

**Materials and Methods:**

Study site

Field sites are located near Chamrousse, Isère, French Alps (45°6′N, 5°54′E). Three mixed, mature, naturally regenerated forests of Norway spruce (*Picea abies* (L.) Karst.), Silver fir (*Abies alba* Mill.), European beech (*Fagus sylvatica* L.) and Mountain pine (*Pinus uncinata* Ramond ex DC.) growing at elevations of 1400 (Prémol), 1700 (Bachat-Bouloud) and 2000 m a.s.l. (near Achard lake, at the treeline) were sampled to assess the effects of changes along elevation for soil physicochemical properties, microbial communities, litter and root traits. Plant species composition shifts from 1400 m to 2000 m with broad-leaved species almost absent above 1700 m. *F. sylvatica* is one of the dominant species at 1400 m and is not present at 1700 and above. *A. alba*, *P. abies*, and *P. uncinata* are the most dominant species at 1400 m, 1700 m, and 2000 m, respectively. *P. abies* is the only species present at all elevations (Mao et al. 2015; Wang et al. 2018b). At the three sites, the forest shows spatial heterogeneity with closed canopy forests and open canopy areas (>10 m diameter) formed through tree mortality after disturbances such as storm or tree felling. Vegetation composition in open canopy areas also changes with elevation with *Gallium rotundifolium*, *Lysimachia nemorum* and *Luzula nivea* being the most dominant species at 1400 m and *Rhododendron ferrugineum* and *Vaccinium myrtillus* at 1700 and 2000 m (Mao et al. 2015; Prieto et al. 2015).

As species composition changed along the elevation gradient, a sampling design comparing closed forest and gaps at each site was selected. This approach is a mean of standardization of the effect of vegetation through the comparison between closed forest and gaps along the elevational

155 gradient. This patch-gap mosaic approach has been used to discern between abiotic and biotic
156 mechanisms underlying the coexistence of phylogenetically related bacteria in a dryland
157 environment (Goberna et al. 2014) but to our knowledge, has never been used to study microbial
158 communities along environmental gradients. A detailed description of selected sites can be found
159 in Wang et al. (2018a) and Mao et al. (2015) and a complete description of species and abundance
160 of herbs and trees in closed forest and gaps at the three elevations can be found in Table S1.

161 The study sites possess similar soil type, and differences between the sites are mainly caused by
162 climatic conditions and the type of vegetation. Soils are acidic at all sites, ranging from (a)
163 "Cambisols (Hyperdystric)" according to the World Reference Base for Soil Resources (IWG
164 2007), above green schist and with an abundant water supply at 1400 m, to (b) "Cambisols
165 (Humic, Hyperdystric)", above the crystalline formation at 1700 m, and to (c) "Epileptic
166 Umbrisols (Hyperdystric)", above the crystalline formation at 2000 m (Joud 2006; Mao et al.
167 2012). More details on the study site are available in Mao et al. (2013, 2015) and Wang et al.
168 (2018b).

169 The air and soil temperatures (10 cm depth) in two closed forests and two gaps were assessed in
170 the three elevations from September 28$^{th}$, 2010 to March 3$^{rd}$, 2014 (Table S2, Fig. S2) with a
171 portable thermistor thermometer (HI-93510N Hanna Instruments) in soil trenches adequately
172 covered by insulation. Additionally, data from Wang et al. (2018a) were used to include soil water
173 potential ($\psi$) in the microclimate and climate assessment (Table S2). These authors used electrical
174 resistance blocks (WaterMark, IRROMETER Company, Inc., USA) installed in one gap and one
175 closed forest at 1400 and 1700 m (but not at 2000 m as equipment was stolen) from September
176 10$^{th}$, 2012 to November 18$^{th}$, 2013.

177 Sampling and storage

178 In each location, three different paired plots (gap *versus* closed forest) with representative patches
179 of closed forests and gaps were chosen (Fig. 1). The conditions for the selection of these pairs
180 were: (i) in closed forest, at least three adult trees of the dominant canopy species were present in

a cluster of trees that had a diameter >8 m, and (ii) gaps comprised an open area with no trees and a diameter greater than 8 m. In closed forests, samples were taken from between trees whilst in gaps, samples were taken in the middle of the gap in order to avoid as much as possible the influence surrounding forest. Four different surface soil samples (0-10 cm) were collected in each plot using sterilised material: (i) a soil cylinder for fine root analysis, (ii) a plastic bag with 10 to 20 g of soil for microbial analysis, (iii) a sample of leaf debris from the surface (hereafter termed 'litter') and (iv) one 0.75 $dm^3$ container of soil for measurements of aggregate stability. A total of 18 samples of each type were collected: 3 elevations x 6 samples at each site three in gaps and three in closed forests). Of each of these 18 samples collected for root analysis, 3 sample fractions were separated: root, rhizosphere and bulk soil making a total of 54 samples. Soil samples were collected during July $7^{th}$ - $10^{th}$, 2014, at the peak of the summer season when microbial activity at the three elevations is expected to be at its maximum.

Soil samples for root, litter and microbial analysis were kept in a freezer at -20ºC until analyses were performed. Soil samples for aggregate stability tests were air-dried in the laboratory until they were processed approximately a month after they were collected.

Soil physicochemical properties

Soil was sieved at 2 mm after air drying and the soil fraction <2 mm was used to assess physicochemical properties. Soil samples were sent to Natural Resource Management (Berkshire, UK). Soil pH was measured in water as 1:2.5 extract. Soil organic matter content was determined via loss-on-ignition at 500 °C (Dean 1974). Total nitrogen (N) and carbon (C) were determined via the DUMAS method (Shea and Watts 1939). Available potassium (K) and magnesium (Mg) were determined through ammonium nitrate extraction and available phosphorus (P) was measured via Olsen (extraction on 0.5 M sodium bicarbonate, (Olsen et al. 1954)). Soil texture was determined by laser-diffraction analysis (McCave et al. 1986). The soil sample was previously digested in hydrogen peroxide solution to destroy the organic matter and sodium hexametaphospate to release the bound clay particles.

Aggregate stability was determined by the fast wetting standard method, ISO/CD 10930, developed by Le Bissonnais (1996). This methodology is appropriate to compare the behaviour of a large range of soils during rapid wetting mimicking heavy rainstorms in summer. Initially, 5 g of aggregates (3-5 mm) were gently immersed in 50 ml of deionized water for 10 min; water was then removed with a pipette and the soil material was transferred to a 50-µm sieve previously immersed in ethanol. The 50 µm sieve immersed in ethanol was gently moved five times to separate fragments smaller and bigger than 50 µm. The >50 µm fraction was collected, oven-dried and gently dry-sieved by hand on a column of six sieves: 2000, 1000, 500, 200, 100 and 50 µm. The mass percentage of each size fraction was calculated, and the aggregate stability was expressed by computation of the mean weight diameter (MWD).

Fine roots and litter

Soil cylinders and litter samples were defrosted. Litter samples were gently washed and rinsed with deionised water and dried at 40ºC until constant weight. Root samples were also washed gently with deionised water and divided in two subsamples: 1) a representative subsample that was selected for scanning and later drying in the oven at 40ºC (n = 18) and the 2) remaining root material of the sample that was dried at 40ºC until constant weight (n = 18). Both subsamples were weighed after drying.

Roots selected for morphological measurement were stained with methylene blue (1 g $L^{-1}$) to increase the contrast and allow the detection of fine roots. Then, roots were placed in a tray with deionised water and scanned (Epson© V700 perfection) at a resolution of 1200 dpi. Analysed roots were then recovered, and oven dried at 40 °C and weighed to obtain dry mass. Root images were analysed with the WinRhizo® software (Pro version 2007, Regent Instrument, Quebec, Canada) using the automatic thresholding option and Lagarde's mode with a filter identifying roots when length was five times the width. Total root length and the length of roots in seven diameter classes (width 0 mm to 1 mm in 0.2 mm classes and 1 mm to 2 mm in 0.5 mm classes) were measured with the software. Specific root length (SRL) was calculated as the ratio between

233 total root length and root dry mass. The percentages of very fine (VFR, diameter < 0.2 mm) and
234 fine (FR, 0.2 < diameter < 1 mm) roots were defined as the ratio of length in the concerned root
235 classes to total root length (Miller and Jastrow 1990). Total root mass density (RMD) was
236 calculated by the ratio of total root dry mass and the soil volume extracted. Total root length
237 density (RLD) was calculated by the ratio of total root length and the soil volume extracted. Total
238 root dry mass was calculated as the sum of the dry mass of roots selected for morphological
239 analysis and those of the remaining roots. Root dry matter content (RDMC) was calculated as the
240 ratio of the root dry weight and root fresh weight.

241 The concentrations of water soluble compounds (cellulose and lignin; mg $g^{-1}$) in root and litter
242 samples (n = 18 for litter and n = 6 for roots as replicates were combined due to limited sample
243 amount), were obtained by the Van Soest Method (1963) with a Fibersac fibre analyser (Ankom,
244 Macedon, USA). Root and litter C and N concentrations (n = 18 for each) were measured using
245 an elemental analyser (Thermo-Finnigan EA1112, Milan, Italy).

246 Soil microbial communities

247 The protocol for cleaning roots and obtaining root, rhizosphere and bulk soil samples was
248 performed following Bulgarelli et al. (2012, 2015). Briefly, loose soil was manually removed
249 from the root system and stored as the bulk soil sample. Roots were collected in 50 ml falcon
250 tubes containing 10 ml PBS-S buffer (130 mM NaCl, 7 mM $Na_2HPO_4$, 3mM $NaH_2PO_4$, pH 7.0,
251 0.02 % Silwet L-77) and washed for 20 minutes at 180 rev $min^{-1}$ on a shaking platform. These
252 roots were transferred to a new falcon tube and subjected to a second washing treatment (20
253 minutes at 180 rpm in 3 ml PBS-S buffer). The soil suspensions collected in the falcon tubes after
254 the first and second washing treatments were combined, centrifuged at 4000g for 20 min and the
255 pellet, considered as the rhizosphere sample, was frozen and stored at -20°C until further
256 processing. Double-washed roots were then transferred to a new falcon tube with 3 ml PBS and
257 sonicated for 2 minutes at 160 W to enrich for microbes living in close association with root
258 tissues. Roots were removed from PBS-S, rinsed in a fresh volume of 10 ml PBS-S buffer and

ground with a mortar and pestle in liquid nitrogen. Pulverised roots (considered as the 'root' sample) were collected in 15 ml falcon tubes and stored at -20°C until further processing.

Total DNA was extracted from soil (0.25 g) and the rhizosphere and root fractions (0.25 g when possible and the entire material available when quantity was less than 0.25 g). DNA extraction was performed using PowerSoil®-htp96 Well Soil DNA Isolation Kit according to manufacturer's instructions (MOBIO Laboratories, UK).

Bacterial and fungal community biodiversity was assessed using Illumina amplicon sequencing of 16S rRNA genes (bacteria) and the Internal transcribed spacer (ITS) region (fungi) to phylogenetically identify responsive taxa. A phylogenetic analysis was also performed for bacterial communities. Amplicon libraries were constructed according to the dual indexing strategy of Kozich et al. (2013), with each primer consisting of the appropriate Illumina adapter, an 8-nt index sequence, a 10-nt pad sequence, a 2-nt linker and the gene specific primer. For 16S, the V3-V4 hypervariable regions of the 16S rRNA gene was targeted using primers based upon the universal primer sequence 341F and 806R. For ITS, region 2 (ITS2) was amplified utilising the fITS7 (forward) and ITS4 (reverse) primer sequences described in Ihrmark et al. (2012). Additional methodological details of Illumina sequencing are described in Notes S1.

Sequenced 16S rRNA paired-end reads were joined using PEAR (Zhang et al. 2014), quality filtered using FASTX tools (hannonlab.cshl.edu), length filtered with the minimum length of 300bps, presence of PhiX and adapters were checked and removed with BBTools (jgi.doe.gov/data-and-tools/bbtools/), and chimeras were identified and removed with VSEARCH_UCHIME_REF (Rognes et al. 2016) using Greengenes Release 13_5 (at 97%) (DeSantis et al. 2006b). Singletons were removed and the resulting sequences were clustered into operational taxonomic units (OTUs) with VSEARCH_CLUSTER (Rognes et al. 2016) at 97% sequence identity (Tindall et al. 2010). Representative sequences for each OTU were taxonomically assigned by RDP Classifier with the bootstrap threshold of 0.8 or greater (Wang et al. 2007) using the Greengenes Release 13_5 (full) (DeSantis et al. 2006b) as the reference.

285 Unless stated otherwise, default parameters were used for the steps listed. ITS2 sequences were
286 processed using the PIPITS pipeline (Gweon et al. 2015), where OTUs were taxonomically
287 assigned against the UNITE database (Release 31.01.2016, Koljalg et al. (2013).

288 Statistical analysis

289 A four-step statistical procedure was performed to determine the interrelationships between the
290 different variables under study: (i) examine the variations on environmental variables (soil
291 properties and root and litter traits) in closed forest versus gaps along the elevational gradient; (ii)
292 non-metric multi-dimensional scaling (NMDS) data ordinations of microbial data and test of the
293 effect of closed forest versus gaps and sample fraction (root, rhizosphere and bulk soil) on their
294 structure (Permutational Multivariate Analysis of Variance, PERMANOVA) along the
295 elevational gradient; (iii) study of correlation between microbial community structure and
296 environmental variables (Spearman correlations and distance-based redundancy analysis,
297 dbRDA); and (iv) study of taxonomic and phylogenetic diversity and divergence among closed
298 forest and gaps along the elevation for the three sample fractions (bulk soil, rhizosphere and root).

299 Air and soil temperatures, soil physicochemical properties and root traits were analysed by
300 Analysis of Covariance (ANCOVA) and Student's t-tests for closed forests *versus* gaps (hereafter
301 termed “tree-gap”). The tree-gap explanatory variable was treated as a factor and elevation was
302 included as a covariate. All variables tested fulfilled ANCOVA assumptions except for SRL that
303 was transformed and RLD, soil P, litter hemicellulose N, C, and C:N that were analysed by
304 Kruskal Wallis tests and Wilcoxon Rank Sum Tests. Soil water potential data were analysed with
305 Friedman rank sum tests and post hoc comparisons were performed using Nemenyi multiple tests.

306 Similarities/dissimilarities between microbial communities were displayed using NMDS of Bray–
307 Curtis dissimilarity for bacterial and fungal OTUs matrices. To indicate similarities between
308 treatments on the NMDS configuration, the points on the NMDS ordination were overlapped with
309 polygons and spider diagrams indicating elevation, tree-gap and soil fraction. PERMANOVA
310 tests (adonis R function) were performed for the bacteria and fungi OTUs matrices as the response

311 and the three different factors (tree-gap and soil fraction) as the explanatory variables and
312 elevation as a covariate.

313 The relationships between the microbial community composition and the soil physicochemical
314 variables, root and litter traits were tested using Spearman correlations between these variables
315 and alpha (Shannon diversity index) and beta diversities (NMDS first and second axis) and a db-
316 RDA. In order to select the environmental parameters to be included in the constrained ordination,
317 an initial db-RDA including all parameters was performed followed by a stepwise model selection
318 using Generalized Akaike Information Criterion (AIC, ordistep function with a backward
319 direction). Finally, the db-RDA analysis was performed only for the variables obtained. ANOVA
320 tests were performed on the final constrained ordination to confirm that the first two axes and the
321 environmental variables and the final constrained ordination were significant.

322 Additionally, the dbRDA analyses were computed for each of the sample fractions and each of
323 the three environmental matrices (soil, root and litter traits) to study the variance explained by
324 these environmental variables and the bacterial and fungal composition.

325 Apart from the traditional taxonomic approach, a phylogenetic approach was also used as it is a
326 useful method to measure biodiversity incorporating phylogenetic difference between species
327 (Webb 2000). The phylogenetic approach relies on homologous genes (genes that are derived
328 from a common ancestor). Here, we sequenced the ITS2 region for fungi which is not a
329 homologous gene, and 16S rRNA gene for bacterial communities (which is homologous).
330 Therefore, the phylogenetic approach was only used for bacterial communities. A phylogenetic
331 tree was constructed based on 16S rRNA representative sequences. The sequences were aligned
332 with PyNAST (Caporaso et al. 2010) to the Greengenes reference database (DeSantis et al.
333 2006a). FastTree (Price et al. 2010) was then used on the resulting alignment to produce a
334 maximum-likelihood phylogenetic tree by iterative rearrangement of branches with generalized
335 time-reversible (GTR) models of nucleotide evolution. This phylogenetic tree was used to
336 generate the distance matrices and obtain two main indexes: one to measure phylogenetic

diversity (Faith´s Phylogenetic Diversity (Faith (1992), hereafter PD) and a second one to measure phylogenetic divergence, the standardized mean nearest taxon distance (hereafter ses.MNTD). Faith´s Phylogenetic Diversity is a measure of the total phylogenetic branch length that joins the basal node to the tips of all species in the sample. Mean nearest taxon distance (MNTD) is an estimate of the mean phylogenetic relatedness between each OTU in a bacterial community and its nearest relative. The standardized mean nearest taxon distance (ses.MNTD) can be used to test for phylogenetic clustering or over dispersion as it is an index that compensates for random processes in the observed phylogenetic community structure. The standardized effects of MNTD were obtained comparing the observed phylogenetic relatedness to the pattern obtained by community randomizations holding community species richness constant (runs = 999, iterations = 1000). Finally, Analysis of Covariance (ANCOVA) tests were performed for these indexes as response variables, tree-gap and sample fraction as explanatory variables and elevation as a covariate. The variables H, PD and ses.MNTD were transformed with a Johnson, inverse and Tukey's Ladder of Powers transformations to meet ANCOVA assumptions.

All analyses were performed in RStudio Version 1.0.136 (RStudio Team 2016) using the *vegan* (Oksanen et al. 2016), *picante* (Kembel et al. 2010), and *ade4* (Dray and Dufour 2007) packages.

**Results**

Relationships between environmental variables and bacterial and fungal communities.

The results of the relationships between soil physicochemical properties, root and litter traits (Tables S3 and S4, Figure 2) and microbial community composition are shown in the db-RDA (Figure 3) and Spearman correlations with alpha and beta diversities (Table 2). The final db-RDA analysis (Figure 3) shows only the environmental parameters that were selected through stepwise model selection. ANCOVA tests performed on the final constrained ordination confirmed that the first two axes, the environmental variables and the final constrained ordination were significant. Bacterial alpha diversity was positively correlated with SRL and negatively correlated with RDMC and root N (Table 2). Additionally, bacterial beta diversity was positively correlated with

363 soil C:N, MRD and root C and negatively correlated to SRL, VFR, RLD and litter hemicellulose.
364 There was a clear separation between closed forests and gaps for bacteria (Figure 3), with
365 aggregates from closed forests having greater stability (MWD), higher root dry matter content
366 (RDMC), soil C and C:N. Gaps were associated to higher VFR and SRL.

367 No correlations were found between any of the parameters measured and the alpha diversity of
368 fungi. Nevertheless, numerous soil properties (sand, SOC, N, C, C:N) and root traits (MRD, N, C
369 and cellulose) were positively correlated with fungal beta diversity whilst other traits were
370 negatively correlated with fungal beta diversity (clay, SRL, VFR, RLD and litter hemicellulose).
371 As for bacterial communities, the composition of fungal communities was different between
372 closed forests and gaps particularly for elevations 1700 and 2000 but not for 1400 m (Figure 3).
373 For fungi, the environmental variables showed similar trends than those obtained for bacteria
374 although more factors were significant (higher MRD, root C, soil N and litter lignin for closed
375 forests and higher RLD, RMD, litter C, N and hemicellulose content for gaps).

376 Data on the climate along the elevation (Table S2, Figure S2) showed that elevation decreased air
377 and soil temperatures and water potential in gaps and closed forests with a gradual decrease of
378 soil and air temperatures for closed forests and a greater decrease between 1700 and 2000 m for
379 gaps. Soil and air temperatures in gaps were higher than in closed forest at the mid elevation (1700
380 m) but not at the lowest and highest elevations (1400 and 2000 m).

381 The dbRDA analyses computed for the sample fractions and environmental matrices (soil, root
382 and litter traits) showed that the variance of bulk soil communities (bacterial and fungal) was
383 mainly explained by soil properties but not by root or litter traits (adjusted $R^2$=0.34 and 0.13
384 respectively, Table 1). Notably, the variance of rhizosphere and root bacterial communities
385 explained by root traits was significant (adjusted $R^2$=0.28 and 0.23 respectively) compared to
386 fungal communities with no significant relationships ($p > 0.05$). Only soil properties significantly
387 explained the variance of soil and rhizosphere fungal communities, with lower explained variance
388 than in bacterial communities (Table 1).

389 Effects of closed forests-gaps and elevation gradient on the structure of bacterial and fungal
390 communities.

391 NMDS ordinations showed that bacterial communities were markedly distinct between closed
392 forests and gaps along the elevation gradient (Figure 4). Conversely, fungal communities did not
393 show this robust distinction between closed forests and gaps, but the elevational effect was
394 evident. These findings were supported by the PERMANOVA performed for the bacterial and
395 fungal species matrices, which showed that the main factors structuring bacterial communities
396 were tree-gap ($R^2 = 0.122$; $p = 0.001$; Table 3) and sample fraction ($R^2 = 0.137$; $p = 0.001$), and
397 the main factors structuring fungal communities were elevation ($R^2 = 0.085$; $p = 0.001$) and tree-
398 gap ($R^2 = 0.062$; $p = 0.001$). Remarkably, sample fraction did not significantly structured fungal
399 communities ($p = 0.163$).

400 Bacterial and fungal composition, diversity and divergence among closed forests and gaps.

401 Most sequences found in the 16S dataset were members of the phyla Proteobacteria,
402 Acidobacteria, Actinobacteria and Verrucumicrobia (Fig. S1). Within the Proteobacteria,
403 Alphaprotebacteria was highly abundant in the three fractions. For fungal communities (ITS
404 region), the sequences most predominant were the Basidiomycota and Ascomycota (Fig. S1).
405 Agaricomycetes was particularly dominant across the three soil fractions and showed a slightly
406 higher presence in bulk soil when compared to rhizosphere and root fractions.

407 Tree-gap ($p = 0.003$) and elevation ($p < 0.001$) significantly modified taxonomic diversity
408 (Shannon's diversity index, H) for bacterial communities but not for fungal communities (Table
409 4, Figure 5). Namely, bacterial H was higher in gaps than in closed forests, and H in soil and
410 rhizosphere communities noticeably increased from 1700 to 2000 m while H in root communities
411 increased from 1400 to 1700 m. Tree-gap ($p < 0.001$) and elevation ($p = 0.033$) significantly
412 modified bacterial Phylogenetic diversity (PD) for bacterial communities. More specifically, a
413 lower PD was found in closed forest ($p < 0.001$; Figure 5). Regarding phylogenetic divergence,
414 bacterial communities showed negative values indicating phylogenetic clustering (Figure 5) in all

situations. Tree-gap modified significantly ses.MNTD ($p = 0.030$; Table 4) with a particularly evident effect of closed forests in reducing the bacterial ses.MNTD in rhizosphere and root communities and at higher elevations.

## Discussion

Our results highlight the importance of plant root traits for the structure and diversity of bacterial communities since bacterial community composition was better explained by root traits than for fungi. The significance of canopy heterogeneity (i.e. open versus closed forest patches) on root traits was demonstrated, along with the structuring impact on associated microbial communities.

We hypothesized that microbial community diversity would be higher in gaps than in closed forest due to increased root trait diversity and root density in gaps. These differences in root properties between closed forest and gaps were supported by our results that showed higher SRL and RLD in gaps. The closed forest and gaps influenced significantly both bacterial and fungal community structure and diversity (Table 1, Figures 3, 4). In agreement with our hypothesis, a general increase in bacterial taxonomic diversity was observed in gaps (Table 4) with a marked effect in the endosphere fraction (Figure 5). Besides the effect of root traits on microbial diversity, an increase in soil temperature in gaps is generally positively related to an increase in soil microbial activities (Muscolo et al. 2007). Here, we found that soil temperature in gaps at a depth of 10 cm at 1700 m was higher (mean temperature of 5.27°C in closed forest and 6.68°C in gaps), which could also partially explain the observed increase in taxonomic diversity in gaps. Since microbial communities and roots are major biotic contributors to soil aggregation (Lehmann et al. 2017), we expected a higher soil aggregate stability in gaps. Contrary to our expectations, soil in closed forests had a higher aggregate stability. Thus, our results highlight the importance of other factors for aggregate stability, such as RDMC, soil C and C:N ratio that were significantly higher in closed forest and are important contributors to soil aggregate stability (Gale et al. 2000; Tisdall and Oades 1982).

Although we found significant effects of closed forest and gaps on the structure of fungal communities along the elevational gradient (Figure 2, Table 1), the effect on fungal taxonomic diversity was not significant (Table 4). These results are in line with results from Collins et al. (2018), who found a high spatial variation of fungal diversity and abundance compromising the predictive power of vegetation and soil properties. This non-significant effect of closed forest and gaps along the elevational gradient on fungal taxonomic diversity could be related to (i) plant-microbe interactions and their specificity (Brundrett 2002; Uroz et al. 2016),(ii) the capacity of dispersion of fungi that could make them very variable across all situations and (iii) the complexities of the Fungi kingdom, as it is an extensive category and successional changes in communities could be masked when using diversity indices. Additionally, we acknowledge that we did not distinguish the presence of saprotrophic, ectomycorrhizal and arbuscular mycorrhizal fungi, which could explain the lack of trends observed for fungal communities.

We observed a phylogenetic clustering in all situations for bacterial communities (negative ses.MNTD values, Figure 5). This observed clustering for bacteria agrees with current consensus as previous studies showed that bacterial communities tend to contain lower taxonomic diversity and are more likely to be phylogenetically clustered than expected by chance (Bryant et al. 2008; Horner-Devine and Bohannan 2006). Consistent with our second hypothesis, we found a lower ses.MNTD in closed forest compared to gaps. This finding indicates the existence of phylogenetic clustering in these environments and suggests the presence of environmental conditions that may cause clustering in closed forest compared to gaps. However, we did not observe this lower ses.MNTD in closed forest compared to gaps at 1400 m, which could be related to the dominance of broadleaved species at this elevation (Prescott and Grayston 2013). Similar results were found by Goberna et al. (2014b) in drylands when comparing vegetation patches to bare soil. These authors found that bacterial communities in gaps were phylogenetically clustered compared to vegetation patches. Goberna et al. (2014) suggested that traits related to environmental stress tolerance are conserved under resource limited conditions (gaps in drylands), while under environments with a high availability of resources (vegetation patches) competitive exclusion of

poorly competitive clades becomes significant (Mayfield and Levine 2010). Consequently, these findings agree with our results if we consider that in this study, resource limited conditions could be found in closed forest compared to gaps since vegetation in gaps had thinner roots with lower RDMC and greater SRL, and higher litter C, N and hemicellulose. Elevation altered the effect of patches on the divergence of bacterial communities, that is likely due to the increase in dominant coniferous species in closed forest and the difference in microclimatic conditions at lower elevations. We found an increase of soil C in closed forest at higher elevations related to the lower decomposability of coniferous litter (Jonard et al. 2017). However, we did not observe the expected decrease in N and acidification of the soil (Hornung 1985).

With the three-stratum sampling approach (soil, rhizosphere and root), we have shown the key role of root properties on microbial communities from bulk soil to root endosphere. We have also highlighted the strength of this sampling approach to adequately identify processes or environmental variables affecting community assemblage. We hypothesized that microbial communities from root and rhizosphere would be greatly affected by root traits compared to microbial communities in the soil. The dbRDA analyses (Table 2) demonstrated a strong relationship between root traits and microbial communities for bacteria, showing that the variance of rhizosphere and root bacterial communities was significantly explained by root traits. A study on root traits along a boreal-temperate forest gradient also demonstrated strong relationships between absorptive root morphology and fungal and bacterial communities with better correlations in rhizosphere samples than in bulk soils (Ostonen et al. 2017). Thus, according to our last hypothesis, the influence of root traits in the rhizosphere and endosphere on bacterial communities was higher than for fungal communities. These results were confirmed with Spearman correlations showing that bacterial Shannon diversity was affected by root traits (SRL, RDMC and N content), but this was not the case for fungi. Similar results in elevational gradients were obtained by Ren et al. (2018) who found that plant diversity modified bacterial but not fungal diversities, as was previously suggested by several authors (Shen et al. 2014; Siles and Margesin 2016) indicating that fungi respond to multiple variables (Jarvis et al. 2015; Ren et al. 2018). This

strong association between root traits and bacterial communities, but not for fungal communities, could be related to the limited capacity of dispersion of bacterial communities as compared to fungal communities with their hyphal growth and branching. According to this, we found that several soil properties were correlated with the beta diversity of fungal communities but not for bacterial communities. Besides, we found that root traits were correlated with both bacterial and fungal beta diversities, mainly SRL, VFR, MRD, RLD and C content, highlighting the importance of fine absorptive roots and root C for microbial communities (Bardgett et al. 2014; de Graaff et al. 2010; Liu et al. 2018).

We found a remarkably high variance of both bacterial and fungal communities explained by soil properties in the dbRDA analysis. This result is in agreement with the proposed hierarchy concerning the contribution of soil and plant species on microbial communities' structure and composition (Bulgarelli et al. 2012; Lareen et al. 2016; Philippot et al. 2013). In other words, soil physicochemical properties determine the composition of the soil microbiome, whereas root traits and exudates can gradually alter the soil microbiome (Bever et al. (2012); van der Putten et al. (2013).

## Conclusions

We found that bacterial community composition was better explained by root traits than for fungi. As expected, this interaction between microbial communities and root traits was more intense in communities isolated from rhizosphere and roots than for the bulk soil. In addition, we found that canopy heterogeneity (i.e. closed forest versus gaps) along the elevation gradient structured bacterial and fungal communities and modified bacterial phylogenetic diversity that decreased in closed forest. Finally, we found that bacterial phylogenetic dispersion was higher in gaps along the elevation gradient. Our results highlight the importance of incorporating (i) root traits, (ii) canopy forest heterogeneity (closed forest versus gaps in this case), and (iii) soil-rhizosphere-endosphere sample fractions in studies along environmental gradients for the correct understanding of factors affecting microbial community assemblage. Overall, our findings

highlight the importance of soil properties for bulk soil and rhizosphere microbial communities and the importance of root traits for rhizosphere and root endosphere bacterial communities but not for fungal communities.

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**Author Contribution**

LMM, RIG, AS, YLB designed, carried out the experimental research and analysed the data. HSG, CFB, AO carried out the experimental research and analysed the data. LMM, RIG, AS wrote the manuscript. All authors edited the manuscript.

**Supporting Information**

**Fig. S1.** Phylum and class of bacterial and fungal communities along the elevation and in gaps and closed forest.

**Fig. S2**. Soil temperature data in gaps and closed forest, over time and for the three elevations.

**Table S1** Dominant species and abundance of herbs and trees in gaps and closed forest along the elevation gradient.

**Table S2** Climate and microclimate data along the elevation gradient in gaps and closed forest.

**Table S3** Soil physicochemical properties along the elevation gradient in gaps and closed forest.

**Table S4** Root and litter traits along the elevation gradient in gaps and closed forest.

**Table S5.** Abbreviations used in this paper

**Notes S1** Additional methodological details on Illumina amplicon sequencing of 16S rRNA genes and the ITS region.

**Tables:**

**Table 1.** Partition of variance in constrained ordination distance-based Redundancy Analysis (dbRDA) for the three sample fractions and the three set of environmental variables (soil properties, root and litter traits). The degrees of freedom (Df), proportion of the variance explained by each model ($R^2$), adjusted $R^2$ (Adj. R2) and its significance (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant) are shown.

| Fraction | | Bulk soil | | | | Rhizosphere | | | | Root | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Df | $R^2$ | Adj.$R^2$ | | Df | $R^2$ | Adj.$R^2$ | | Df | $R^2$ | Adj.$R^2$ | |
| **Soil properties** | **Bacteria** | 11 | 0.79 | 0.34 | ** | 11 | 0.81 | 0.39 | ** | 11 | 0.76 | 0.24 | * |
| **Root traits** | | 10 | 0.66 | 0.10 | ns | 10 | 0.73 | 0.28 | * | 10 | 0.71 | 0.23 | * |
| **Litter traits** | | 6 | 0.40 | 0.04 | ns | 6 | 0.45 | 0.12 | ns | 6 | 0.39 | 0.02 | ns |
| **Soil properties** | **Fungi** | 11 | 0.73 | 0.13 | * | 11 | 0.70 | 0.15 | *** | 11 | 0.80 | 0.07 | ns |
| **Root traits** | | 10 | 0.65 | 0.06 | ns | 10 | 0.61 | 0.06 | ns | 10 | 0.71 | 0.00 | ns |
| **Litter traits** | | 6 | 0.39 | 0.02 | ns | 6 | 0.36 | 0.02 | ns | 6 | 0.47 | 0.07 | * |

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**Table 2.** Spearman's correlation coefficients between bacterial and fungal alpha diversity (H: Shannon index) and beta diversity (NMDS1 and NMDS2), and soil properties, root and litter traits (****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05)**.** Refer to Table S5 for abbreviations.

| | | Bacteria | | | Fungi | | |
|---|---|---|---|---|---|---|---|
| | | **H** | **NMDS1** | **NMDS2** | **H** | **NMDS1** | **NMDS2** |
| **Soil** | **Sand** | 0.13 | 0.14 | 0.30* | -0.14 | -0.12 | **0.72****** |
| | **Silt** | -0.2 | 0.1 | -0.23 | -0.05 | 0.30* | -0.49*** |
| | **Clay** | 0.04 | -0.37** | -0.30* | 0.2 | -0.16 | **-0.68****** |
| | **pH** | 0.18 | -0.24 | -0.06 | -0.25 | -0.49*** | 0.17 |
| | **P** | 0.22 | -0.30* | -0.23 | 0.17 | -0.33* | -0.12 |
| | **Potassium** | 0.22 | 0.11 | 0.40** | -0.15 | -0.15 | 0.50*** |
| | **Magnesium** | 0.06 | 0.25 | 0.49*** | -0.07 | 0.19 | 0.41** |
| | **SOC** | -0.03 | 0.43** | 0.41** | -0.34* | 0.18 | **0.64****** |
| | **N** | 0.1 | 0.27 | 0.38** | -0.30* | -0.06 | **0.58****** |
| | **C** | 0.01 | 0.40** | 0.42** | -0.31* | 0.07 | **0.69****** |
| | **C:N** | -0.29* | **0.63****** | 0.25 | -0.16 | 0.38** | **0.55****** |
| **Roots** | **SRL** | **0.50***** | **-0.76****** | -0.06 | -0.02 | **-0.66****** | -0.21 |
| | **VFR** | 0.2 | **-0.53****** | -0.23 | -0.19 | **-0.54****** | -0.28 |
| | **FR** | 0 | 0.08 | 0.2 | 0.16 | 0.19 | 0.09 |
| | **MRD** | -0.45*** | **0.77****** | 0.09 | 0.07 | **0.70****** | 0.21 |
| | **RDMC** | **-0.55****** | 0.37** | -0.17 | -0.2 | 0.44** | -0.13 |
| | **RMD** | 0.1 | -0.25 | -0.17 | -0.23 | -0.37** | -0.05 |
| | **RLD** | 0.47*** | **-0.71****** | -0.1 | -0.06 | **-0.70****** | -0.21 |
| | **N** | **-0.55****** | 0.42** | -0.36** | -0.14 | **0.64****** | -0.36* |
| | **C** | -0.37** | **0.57****** | -0.01 | -0.2 | **0.53****** | -0.02 |
| | **C:N** | 0.39** | -0.21 | 0.43** | 0.09 | -0.42** | 0.42** |
| | **lignin** | -0.15 | 0.17 | 0.19 | -0.42 | -0.05 | 0.21 |
| | **cellulose** | -0.35 | 0.02 | -0.22 | 0.31 | **0.56*** | -0.45 |
| | **hemicellulose** | 0.18 | -0.42 | -0.25 | 0.35 | -0.040 | -0.07 |
| **Litter** | **N** | 0.40** | -0.25 | 0.28* | 0.01 | -0.34* | 0.21 |
| | **C** | 0.25 | 0.05 | 0.29* | 0.09 | 0.06 | 0.22 |
| | **C:N** | -0.25 | 0.24 | -0.15 | 0.02 | 0.28 | -0.04 |
| | **lignin** | 0.15 | 0.07 | 0.27 | -0.24 | -0.01 | 0.22 |
| | **cellulose** | -0.37** | 0.40** | -0.12 | 0.11 | 0.45*** | -0.14 |
| | **hemicellulose** | 0.23 | **-0.62****** | -0.32* | 0.07 | **-0.51***** | -0.35* |

**Table 3.** Effects of tree-gap, elevation and sample fraction and their interactions on the structure of bacterial and fungal communities assessed with PERMANOVA. The degrees of freedom (Df),), sum of squares (sum of sqs), mean of squares (mean of sqs), the F. statistic, the proportion of the variance explained by each model ($R^2$) and probability (P) are shown.

| | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Factors** | **Df** | **Sum of sqs** | **Mean of sqs** | **F** | **$R^2$** | **P(>F)** | **Df** | **Sum of sqs** | **Mean of sqs** | **F** | **$R^2$** | **P(>F)** |
| **Tree-gap** | 1 | 1.0542 | 1.05417 | 8.7707 | 0.122 | 0.001 | 1 | 1.2443 | 1.24429 | 3.3684 | 0.062 | 0.001 |
| **Elevation** | 1 | 0.5703 | 0.57034 | 4.7452 | 0.066 | 0.001 | 1 | 1.7022 | 1.70222 | 4.608 | 0.085 | 0.001 |
| **Samplefraction** | 2 | 1.1862 | 0.59308 | 4.9344 | 0.137 | 0.001 | 2 | 0.8555 | 0.42776 | 1.158 | 0.043 | 0.163 |
| **Elevation:Tree-gap** | 1 | 0.3944 | 0.3944 | 3.2814 | 0.045 | 0.003 | 1 | 0.9148 | 0.91483 | 2.4765 | 0.046 | 0.001 |
| **Elevation:Samplefraction** | 2 | 0.2742 | 0.13708 | 1.1405 | 0.032 | 0.249 | 2 | 0.4946 | 0.24729 | 0.6694 | 0.025 | 0.994 |
| **Tree-gap:Samplefraction** | 2 | 0.2578 | 0.12892 | 1.0726 | 0.030 | 0.34 | 2 | 0.4086 | 0.2043 | 0.553 | 0.020 | 1 |
| **Elevation:Tree-gap:Samplefraction** | 2 | 0.2495 | 0.12474 | 1.0378 | 0.029 | 0.366 | 2 | 0.4484 | 0.22419 | 0.6069 | 0.022 | 0.998 |
| **Residuals** | 39 | 4.6875 | 0.12019 | | 0.540 | | 38 | 14.0373 | 0.3694 | | 0.698 | |
| **Total** | 50 | 8.6741 | | | 1 | | 49 | 20.1057 | | | 1 | |

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**Table 4.** Analysis of covariance (ANCOVA) table showing the effects of tree-gap, elevation and sample fraction on bacterial and fungal taxonomic (H) and phylogenetic (PD) diversities and phylogenetic divergence (ses.MNTD). The degrees of freedom (Df), the F statistic and P values are shown. Refer to Table S5 for abbreviations.

| | | | Bacteria | | Fungi | |
|---|---|---|---|---|---|---|
| | | Df | F | P value | F | P value |
| H | Tree-gap | 1 | 10.25 | 0.003 | 0.048 | 0.829 |
| | Elevation | 1 | 14.89 | <0.001 | 0.02 | 0.889 |
| | Samplefraction | 2 | 0.52 | 0.599 | 3.231 | 0.051 |
| | Elevation:Tree-gap | 1 | 0.432 | 0.515 | 2.102 | 0.155 |
| | Elevation:Samplefraction | 2 | 0.142 | 0.868 | 0.48 | 0.622 |
| | Tree:Samplefraction | 2 | 1.099 | 0.343 | 1.524 | 0.231 |
| | Elevation:Tree-gap:Samplefraction | 2 | 0.559 | 0.576 | 0.049 | 0.952 |
| PD | Tree-gap | 1 | 20.7 | <0.001 | | |
| | Elevation | 1 | 4.879 | 0.033 | | |
| | Samplefraction | 2 | 0.479 | 0.623 | | |
| | Elevation:Tree-gap | 1 | 0.141 | 0.710 | | |
| | Elevation:Samplefraction | 2 | 0.257 | 0.775 | | |
| | Tree:Samplefraction | 2 | 1.075 | 0.351 | | |
| | Elevation:Tree-gap:Samplefraction | 2 | 0.55 | 0.582 | | |
| ses.MNTD | Tree-gap | 1 | 5.106 | 0.030 | | |
| | Elevation | 1 | 1.864 | 0.180 | | |
| | Samplefraction | 2 | 3.117 | 0.056 | | |
| | Elevation:Tree-gap | 1 | 1.681 | 0.202 | | |
| | Elevation:Samplefraction | 2 | 0.506 | 0.607 | | |
| | Tree:Samplefraction | 2 | 1.237 | 0.301 | | |
| | Elevation:Tree-gap:Samplefraction | 2 | 0.033 | 0.968 | | |

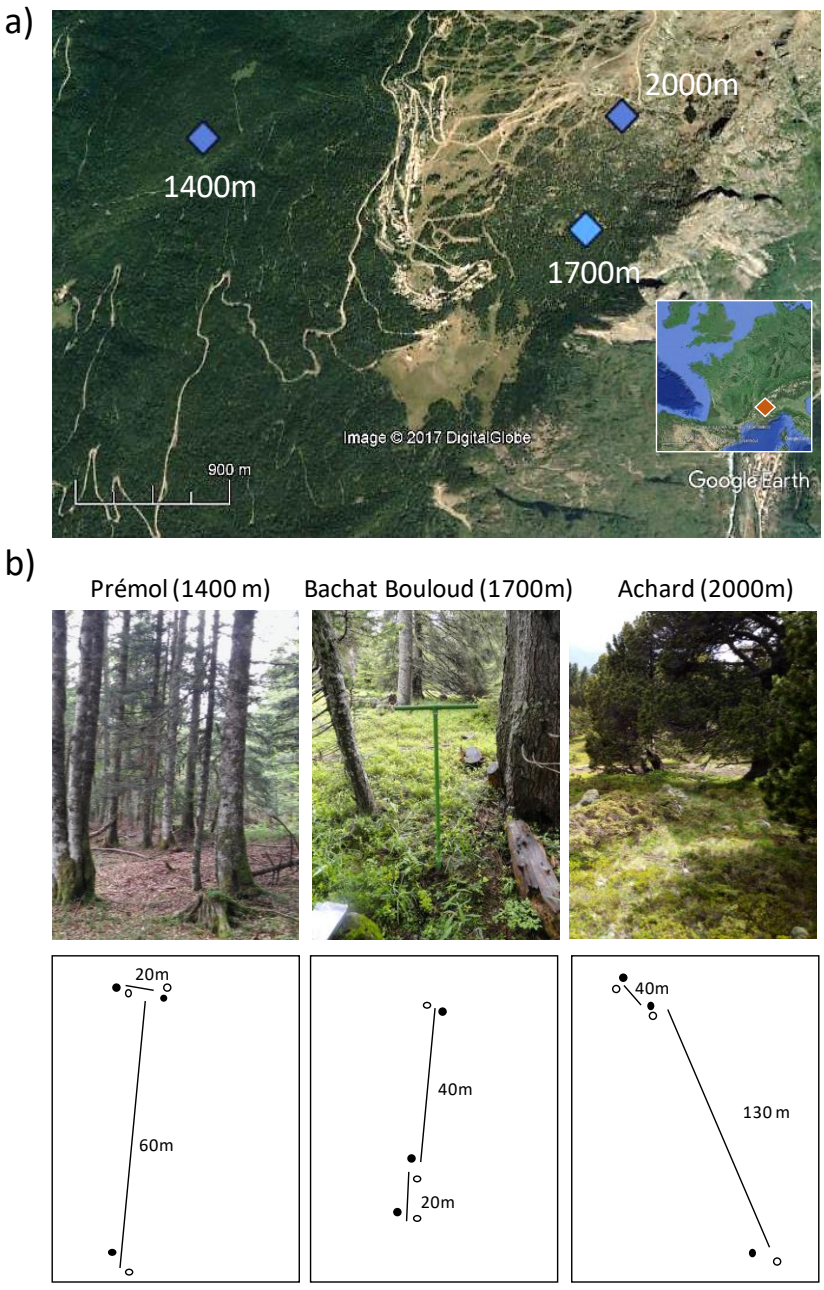


**Figure 1.** a) Location of the plots in the three elevations (1400, 1700 and 2000m) where samples were taken (Map data: Google, Image ©2017 Digital Globe); b) pictures showing the three sites where root, soil and litter were collected for analysis and location of pairs of gaps (open circles) and closed forest (closed circles). Distances (in m), between each plot are indicated.

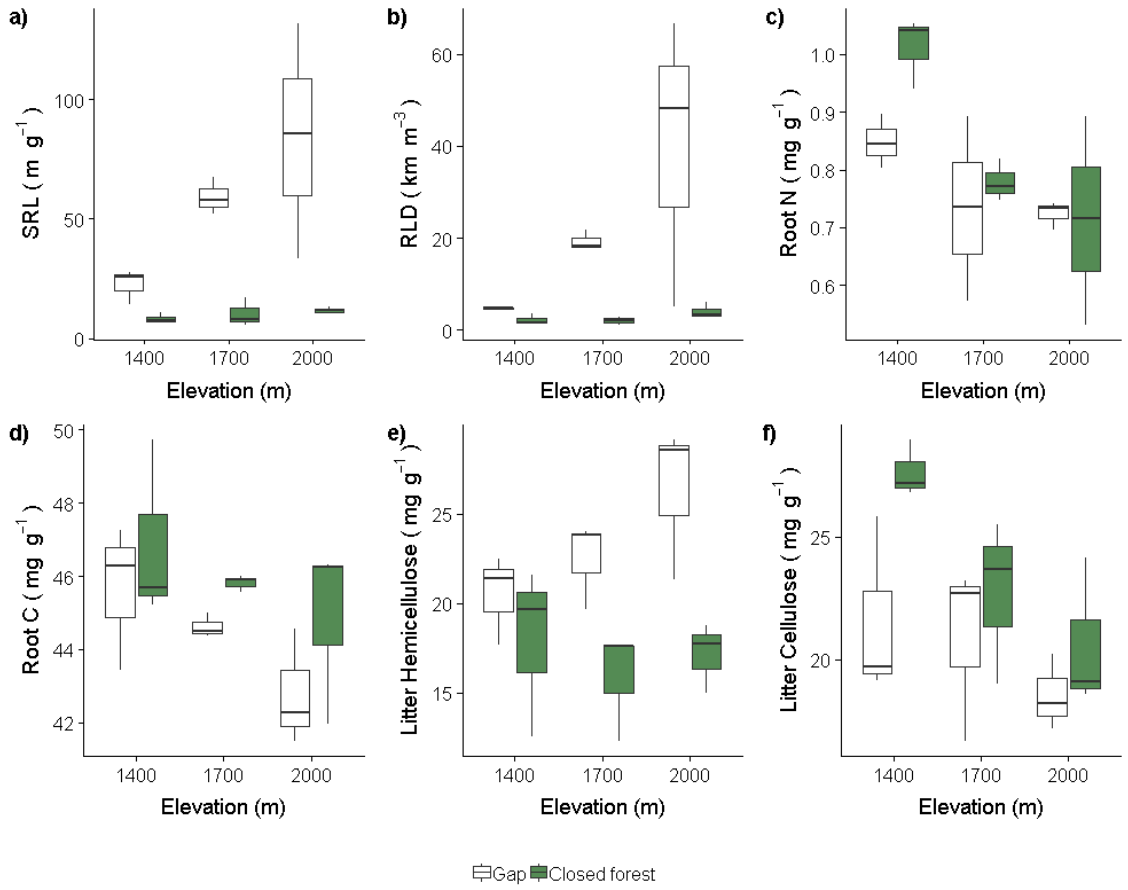


**Figure 2.** A selection of root and litter physical traits: (a) specific root length (SRL), (b) root length density (RLD); and chemical traits: (c) root nitrogen (N), (d) root carbon (C), (e) litter hemicellulose and (f) litter cellulose in gaps (white bars) and closed forest (green bars) along the elevation gradient. Boxplots represent the minimum, maximum, median, first quartile and third quartile in the data set.

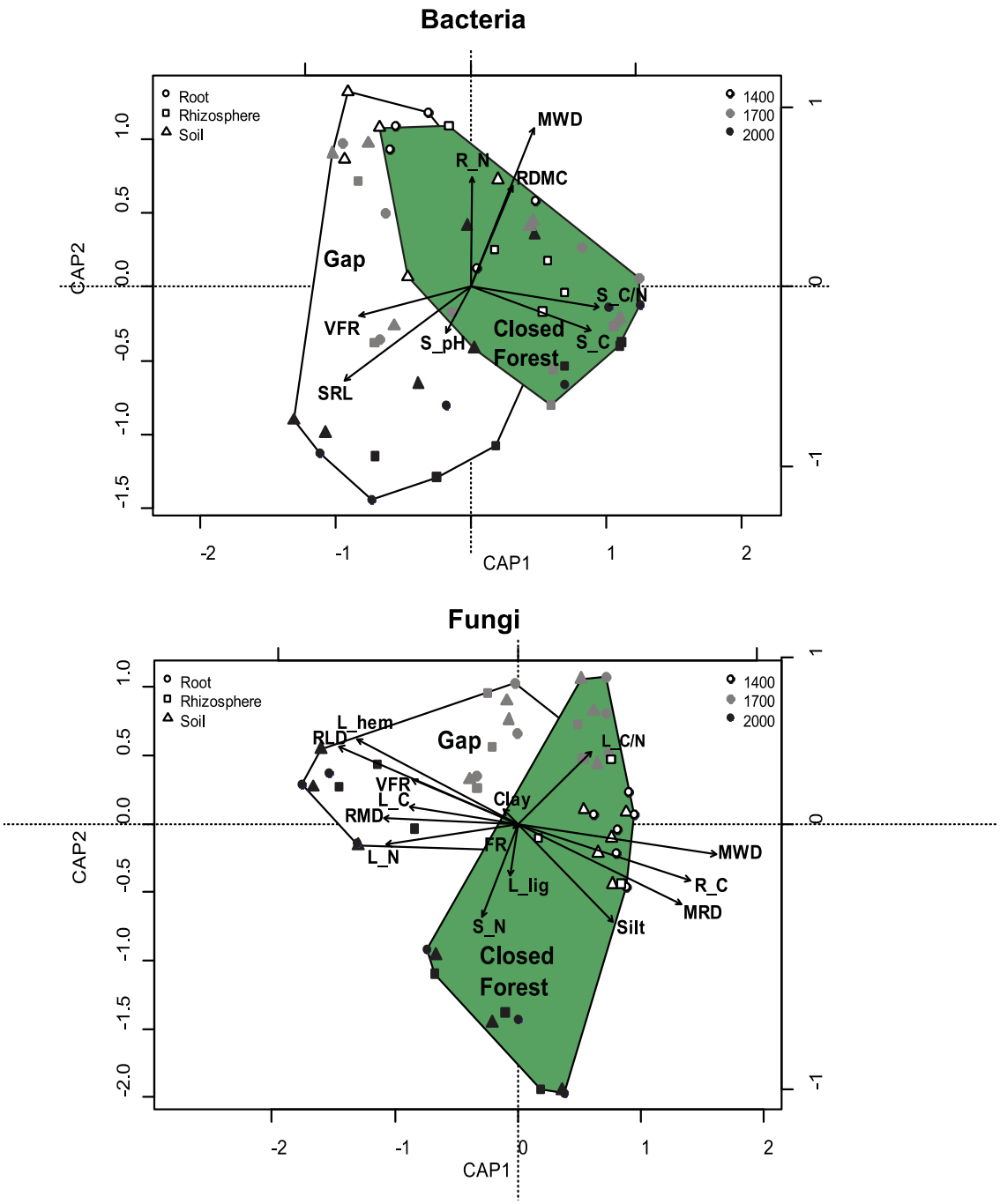


**Figure 3.** Graphs of dbRDA constrained ordinations of bacterial and fungal species matrices with convex hull polygons containing plots of gaps (white) and closed forest (green) and significant variables obtained by automatic backward stepwise model building. Data are shown for roots (○), rhizosphere (□) and bulk soil (△) fractions at 1400 m (white symbols), 1700 m (grey symbols) and 2000 m (black symbols).

**a) Bacteria** **b) Fungi**

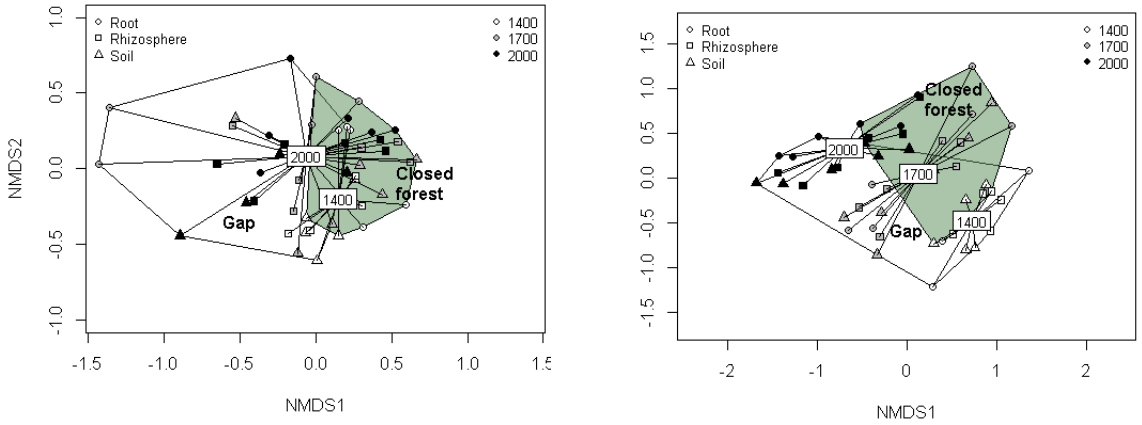


**Figure 4.** NMDS ordinations of a) bacterial and b) fungal species matrices with convex hull polygons containing plots of the two locations (gap and closed forest white and green shaded respectively) and spider diagrams linking plots with the same elevation (2000 and 1700 m tags are overlapped for bacteria). Sample fractions are shown with different symbols (○: root, □: rhizosphere, △: bulk soil).

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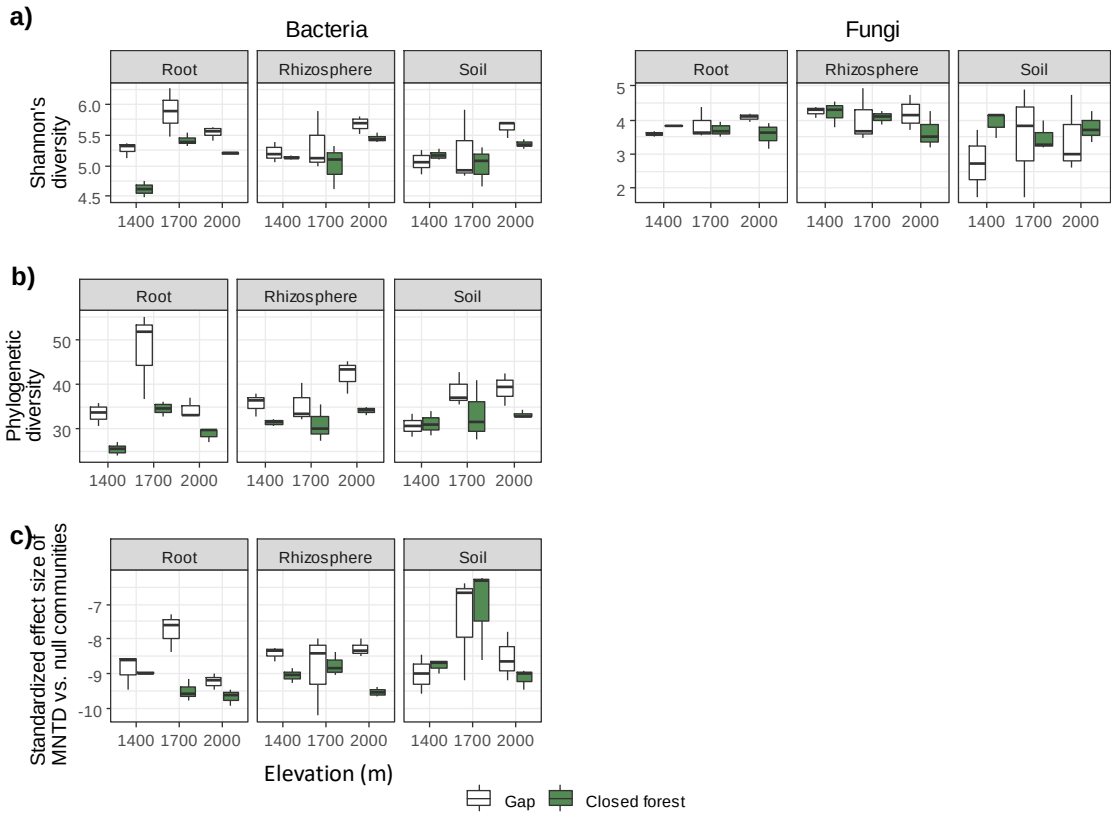


**Figure 5.** Changes in a) bacterial taxonomic diversity (Shannon's diversity), b) phylogenetic diversity and c) phylogenetic clustering (standardized effect size of MNTD versus null communities, ses.MNTD) in gaps (white bars) and closed forest (green bars), along the elevational gradient for the three sample fractions (root, rhizosphere and soil). Boxplots represent the minimum, maximum, median, first quartile and third quartile in the data set.

**Supporting Information**

**Table S1.** Dominant species and abundance of herbs and trees in gaps and closed forest along the elevation gradient. Abundance of herbs and trees is given as a percentage foliar cover. Age to last disturbance (Dist) is the time (in years) to the last disturbance (tree thinning). Taken from Prieto et al. (2015).

| Elevation | Land use type | Dominant species | Herbs (% cover) | Trees (% cover) | Dist (years) |
|---|---|---|---|---|---|
| 1400 | Gap | *Galium rotundifolium* L., *Lysimachia nemorum* L., *Luzula nivea* (Nath.) DC | 89 | 0 | 5 |
| | Closed forest | *Abies alba* Mill., *Picea abies* (L.) H. Karst., *Fagus sylvatica* L. | 10 | 90 | 40 |
| 1700 | Gap | *Luzula nivea* (Nath.) DC *Rhododendron ferrugineum* L., *Vaccinium myrtillus* L. | 55 | 0 | 5 |
| | Closed forest | *Picea abies* (L.) H. Karst., *Abies alba* Mill. | 0 | 90 | 40 |
| 2000 | Gap | *Rhododendron ferrugineum* L., *Vaccinium myrtillus* L. | 50 | 0 | 5 |
| | Closed forest | *Pinus uncinata* Ramond ex. DC., *Picea abies* (L.) H. Karst. | 10 | 90 | 40 |

**Table S2.** Climate and microclimate data (mean ± standard error) along the elevation gradient in gaps and closed forest. P-values of analysis of covariance (ANCOVA) tests are shown for air and soil temperatures and Friedman tests for soil water potential. Number of repeated measures is shown between brackets. (***P < 0.001, **P < 0.01, *P < 0.05)**.** Post hoc comparisons performed with Nemenyi multiple tests.

| | 1400 | | | 1700 | | | 2000 | | | Tree | Elevation | Elevation* Tree |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gap | | Closed forest | Gap | | Closed forest | Gap | | Closed forest | p | p | p |
| **Air temperature (°C)** | 8.24 ± 1.04 (68) | ns | 7.73 ± 1.01 (66) | 8.59 ± 1.04 (67) | ** | 7.44 ± 0.93 (66) | 7.33 ± 0.94 (51) | ns | 6.96 ± 0.94 (56) | 0.08 ns | <0.001 *** | 0.91 ns |
| **Soil temperature** 10 cm depth (°C) | 6.48 ± 0.57 (68) | ns | 6.36 ± 0.57 (66) | 6.68 ± 0.63 (67) | * | 5.27 ± 0.46 (66) | 5.64 ± 0.63 (51) | ns | 5.42 ± 0.55 (56) | 0.00: ** | <0.001 *** | 0.36 ns |
| **Soil water potential** 20 cm depth (kpa) | -10.67 ± 2.54 (11) | ns | -59.15 ± 17.31 (11) | -13.88 ± 2.43 (11) | ns | -81.23 ± 24.79 (11) | - | | - | 0.13 ns | 0.37 ns | - |

**Table S3.** Soil physicochemical properties along the elevation gradient in gaps and closed forest. p-values from analysis of covariance (ANCOVA) tests are shown. Two sample t-test significant results at $p < 0.05$ are shown with the symbol "+" for gap versus closed forest. Kruskal-Wallis and Wilcoxon test were performed for p. Refer to Table S5 for abbreviations.

| | 1400 | | 1700 | | 2000 | | Tree | Elevation | Elevation*Tree |
|---|---|---|---|---|---|---|---|---|---|
| | Gap | Closed forest | Gap | Closed forest | Gap | Closed forest | p | p | p |
| **MWD (mm)** | 3.37 ± 0.03 | 3.35 ± 0.02 | 3.33 ± 0.02 | 3.33 ± 0.03 | 3.02 ± 0.03 | 3.31 ± 0.03 + | 0.102 | 0.008 | 0.028 |
| **pH** | 4.43 ± 0.09 | 4.80 ± 0.13 | 5.03 ± 0.11 | 4.90 ± 0.08 | 5.27 ± 0.07 | 5.03 ± 0.07 | 0.900 | 0.022 | 0.147 |
| **P (mg/l)** | 4.60 ± 0.50 | 5.47 ± 0.35 | 3.13 ± 0.09 | 2.73 ± 0.12 | 9.40 ± 0.62 | 3.73 ± 0.09 | 0.148 | 0.005 | - |
| **Potassium (mg $l^{-1}$)** | 30.73 ± 5.66 | 31.80 ± 1.33 | 44.97 ± 5.27 | 50.77 ± 2.68 | 49.43 ± 3.64 | 63.53 ± 6.02 | 0.465 | 0.022 | 0.238 |
| **Magnesium (mg $l^{-1}$ )** | 44.53 ± 5.75 | 33.57 ± 3.48 | 69.43 ± 6.71 | 94.20 ± 6.83 | 32.37 ± 3.23 | 67.60 ± 5.80 | 0.167 | 0.416 | 0.250 |
| **Sand (% w $w^{-1}$)** | 35.67 ± 2.13 | 44.00 ± 0.29 | 40.67 ± 0.83 | 58.33 ± 2.19 + | 53.67 ± 1.59 | 46.67 ± 0.33 | 0.133 | 0.066 | 0.117 |
| **Silt (% w $w^{-1}$)** | 35.67 ± 0.73 | 31.67 ± 0.17 | 31.00 ± 0.50 | 24.00 ± 1.26 | 24.33 ± 0.73 | 31.00 ± 0.58 + | 0.571 | 0.037 | 0.039 |
| **Clay (% w $w^{-1}$)** | 28.67 ± 1.42 | 24.33 ± 0.44 | 28.33 ± 0.33 | 17.67 ± 0.93 + | 22.00 ± 0.87 | 22.33 ± 0.88 | 0.031 | 0.171 | 0.404 |
| **SOC (% w $w^{-1}$)** | 12.00 ± 0.32 | 11.53 ± 1.53 | 15.33 ± 0.40 | 22.70 ± 0.77 + | 12.63 ± 1.86 | 22.17 ± 1.29 | 0.028 | 0.081 | 0.087 |
| **N (% w $w^{-1}$)** | 0.34 ± 0.01 | 0.33 ± 0.05 | 0.47 ± 0.01 | 0.50 ± 0.01 | 0.42 ± 0.07 | 0.64 ± 0.03 | 0.185 | 0.024 | 0.097 |
| **TC (% w $w^{-1}$)** | 5.37 ± 0.12 | 5.53 ± 0.75 | 7.47 ± 0.16 | 11.37 ± 0.42 + | 6.37 ± 0.92 | 12.43 ± 0.16 + | 0.004 | 0.010 | 0.029 |
| **C : N** | 15.71 ± 0.44 | 16.70 ± 0.10 + | 15.97 ± 0.16 | 22.70 ± 0.64 + | 15.33 ± 0.17 | 19.50 ± 0.64 + | 0.002 | 0.380 | 0.346 |

**Table S4.** Root and litter traits along the elevation gradient in gaps and closed forest. P-values of analysis of variance (ANOVA) tests or Kruskal Wallis are shown. Different letters show post-hoc Tukey honestly significant difference (HSD) results for elevation. Two sample t-test significant results at $p < 0.05$ are shown with the symbol "+" for gap versus closed forest. Refer to Table S5 for abbreviations.

| | 1400 | | 1700 | | 2000 | | Tree | Elevation | Elevation*Tree |
|---|---|---|---|---|---|---|---|---|---|
| | Gap | Closed forest | Gap | Closed forest | Gap | Closed forest | p | p | p |
| **Root traits** | | | | | | | | | |
| SRL (m gr$^{-1}$) | 22.61 ± 4.21 | + 8.16 ± 1.28 | 59.20 ± 4.49 | + 10.36 ± 3.49 | 83.76 ± 28.40 | + 11.66 ± 0.83 | <0.001 | 0.005 | 0.037 |
| VFR (%) | 40.65 ± 1.80 | 49.69 ± 5.30 | 57.86 ± 3.48 | 39.29 ± 6.30 | 54.76 ± 4.05 | 45.41 ± 4.39 | 0.146 | 0.343 | 0.088 |
| FR (%) | 52.01 ± 3.86 | 34.66 ± 5.79 | 40.52 ± 3.30 | 46.83 ± 4.52 | 43.65 ± 3.22 | 44.81 ± 4.67 | 0.393 | 0.847 | 0.063 |
| Mean Root Diameter (mm) | 0.40 ± 0.03 | + 0.53 ± 0.02 | 0.26 ± 0.02 | + 0.52 ± 0.09 | 0.27 ± 0.03 | + 0.46 ± 0.02 | <0.001 | 0.030 | 0.499 |
| RDMC (gr gr$^{-1}$) | 0.37 ± 0.01 | 0.40 ± 0.01 | 0.37 ± 0.04 | 0.39 ± 0.02 | 0.33 ± 0.01 | 0.37 ± 0.02 | 0.076 | 0.138 | 0.623 |
| RMD (g m$^{-3}$) | 228.65 ± 56.37 | 272.16 ± 37.30 | 332.37 ± 44.57 | 211.41 ± 31.25 | 406.38 ± 128.18 | 341.92 ± 65.62 | 0.086 | 0.402 | 0.434 |
| RLD (km m$^{-3}$) | 4.70 ± 0.13 | 2.30 ± 0.67 | 19.30 ± 1.22 | 2.05 ± 0.47 | 40.01 ± 18.25 | 4.05 ± 1.01 | 0.001 | 0.264 | - |
| Lignin (mg g$^{-1}$) | 46.04 | 54.56 | 63.37 | 54.67 | 50.36 | 59.74 | - | - | - |
| Cellulose (mg g$^{-1}$) | 27.90 | 22.61 | 18.75 | 21.82 | 19.61 | 16.16 | - | - | - |
| Hemicellulose (mg g$^{-1}$) | 21.97 | 17.67 | 17.47 | 19.00 | 31.05 | 16.87 | - | - | - |
| N (mg g$^{-1}$) | 0.85 ± 0.03 | + 1.01 ± 0.04 | 0.73 ± 0.09 | 0.78 ± 0.02 | 0.72 ± 0.01 | 0.71 ± 0.10 | 0.200 | 0.003 | 0.170 |
| C (mg/g) | 45.67 ± 1.15 | 46.88 ± 1.43 | 44.63 ± 0.19 | + 45.82 ± 0.14 | 42.78 ± 0.92 | 44.85 ± 1.44 | 0.022 | 0.077 | 0.662 |
| C : N | 54.01 ± 3.03 | 46.39 ± 1.52 | 62.89 ± 8.12 | 58.92 ± 1.41 | 59.13 ± 1.58 | 65.16 ± 7.85 | 0.643 | 0.026 | 0.176 |
| **Litter traits** | | | | | | | | | |
| Lignin (mg g$^{-1}$) | 47.10 ± 2.45 | 45.57 ± 2.66 | 49.11 ± 1.74 | 51.53 ± 3.71 | 48.79 ± 2.81 | 51.21 ± 1.38 | 0.595 | 0.164 | 0.442 |
| Cellulose (mg g$^{-1}$) | 21.57 ± 2.15 | 27.67 ± 0.66 | 20.87 ± 2.11 | 22.73 ± 1.95 | 18.54 ± 0.89 | 20.61 ± 1.79 | 0.023 | 0.007 | 0.231 |
| Hemicellulose (mg g$^{-1}$) | 20.50 ± 1.47 | 17.93 ± 2.74 | 22.49 ± 1.41 | 15.84 ± 1.77 | 26.34 ± 2.52 | 17.16 ± 1.13 | 0.004 | 0.751 | - |
| N (mg g$^{-1}$) | 1.16 ± 0.02 | 0.84 ± 0.06 | 0.95 ± 0.21 | 0.92 ± 0.21 | 1.70 ± 0.53 | 1.12 ± 0.10 | 0.122 | 0.240 | - |
| C (mg g$^{-1}$) | 40.97 ± 1.13 | 38.72 ± 2.18 | 37.93 ± 3.56 | 43.09 ± 1.15 | 52.63 ± 12.92 | 40.41 ± 1.18 | 0.825 | 0.864 | - |
| C : N | 35.44 ± 0.42 | 46.87 ± 5.68 | 44.12 ± 10.09 | 53.96 ± 15.69 | 33.05 ± 4.88 | 36.68 ± 2.56 | 0.058 | 0.386 | - |

548

549 **Table S5.** Abbreviations used in this paper

| Variable | abbreviation | units |
|---|---|---|
| **Soil properties** | | |
| Mean weight diameter | MWD | mm |
| P | Phosphorus | $mg \cdot l^{-1}$ |
| Soil organic carbon | SOC | $\%w \cdot w^{-1}$ |
| Total carbon | TC | %w·w-1 |
| **Root traits** | | |
| Specific root length | SRL | $m \cdot g^{-1}$ |
| Very fine roots | VFR | % |
| Fine roots | FR | % |
| Mean Root Diameter | MRD | mm |
| Root dry matter content | RDMC | $mg \cdot g^{-1}$ |
| Root mass density | RMD | $g \cdot m^{-3}$ |
| Root length density | RLD | $km \cdot m^{-3}$ |
| **General abbreviations** | | |
| Nitrogen | N | $\%w \cdot w^{-1}$ |
| Carbon | C | $\%w \cdot w^{-1}$ |
| C-to-N ratio | C:N | none |
| Shannon's diversity | H | none |
| Faith´s Phylogenetic Diversity | PD | none |
| Standardized mean nearest taxon distance | ses.MNTD | none |

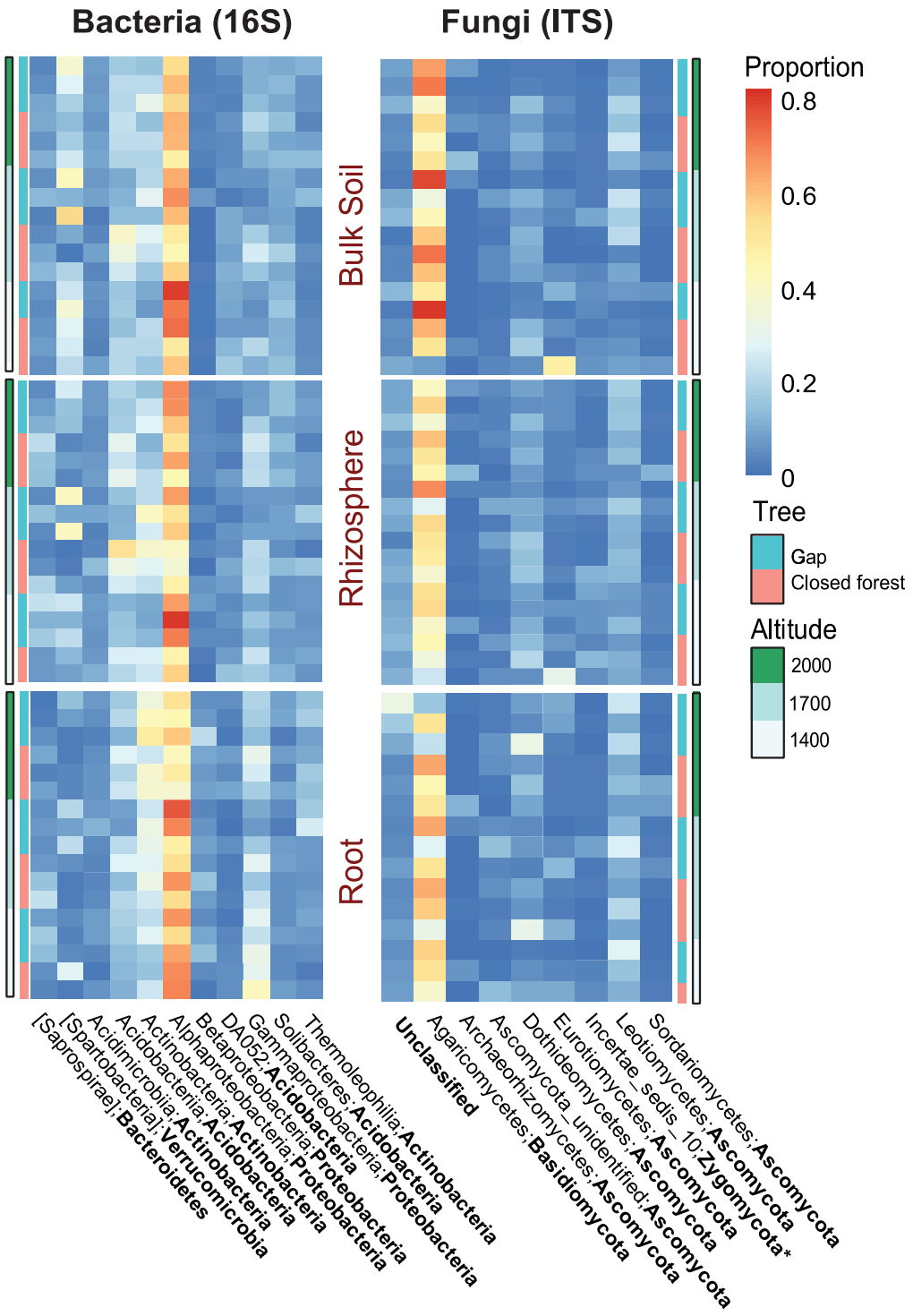


**Fig. S1.** Phylum and class of bacterial and fungal communities along the elevation and in gaps and closed forest. Proportion of presence within sampling unit. Only the most abundant classes are shown (for which the sum of proportions for all sites were greater than one). **Zygomycota* assignation here was before their later reclassification among *Glomeromycota* and several subphyla *incertae sedis* (Hibbett et al. 2007).

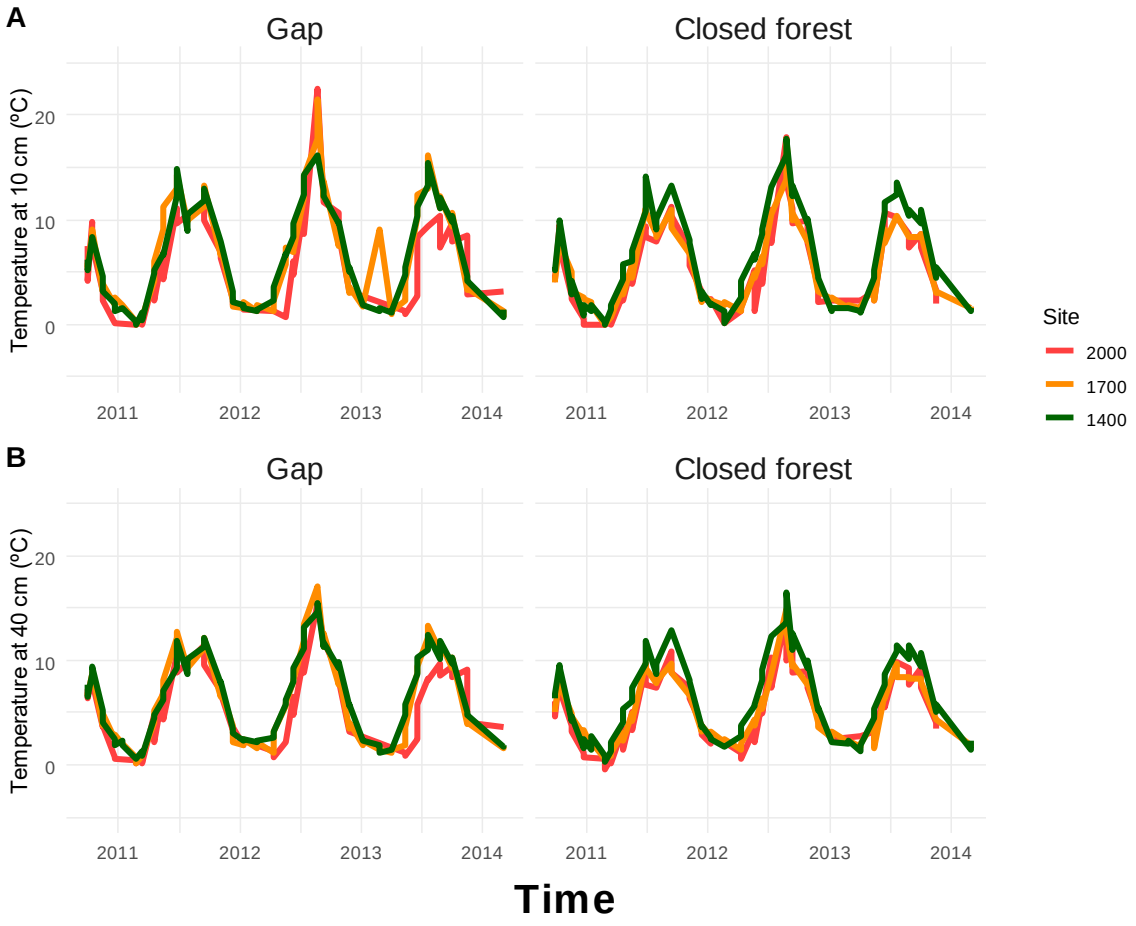


**Fig. S2.** Soil temperature data in gaps and closed forest, over time and for the three elevations: a) soil temperature at a depth of 10 cm and b) soil temperature at a depth of 40 cm.

**Notes S1** Additional methodological details on Illumina amplicon sequencing of 16S rRNA genes and the ITS region.

Amplicons were generated using a high-fidelity DNA polymerase (Q5 Taq, New England Biolabs) and pooled. PCR was conducted on 20 ng of template DNA employing an initial denaturation of 30 seconds at 95 ºC, followed by cycles (25 for 16S and 30 for ITS) of 30 seconds at 95 ºC, 30 seconds at 52 ºC and 2 minutes at 72 ºC. A final extension of 10 minutes at 72 ºC was also included to complete the reaction. Amplicon sizes were determined using an Agilent 2200 TapeStation system (~550bp:16S; ~350-425: ITS; ~650:18S) and libraries normalized using SequalPrep Normalization Plate Kit (Thermo Fisher Scientific). Library concentration was calculated using a SYBR green quantitative PCR (qPCR) assay with primers specific to the Illumina adapters (Kappa, Anachem). Libraries were sequenced at a concentration of 5.4 pM with a 0.6 pM addition of an Illumina generated PhiX control library. Sequencing runs, generating 2 x 300 bp, reads were performed on an Illumina MiSeq using V3 chemistry. The read 1 (R1), read 2 (R2) and index sequencing primers used were also gene specific: R1 = sequence of the combined pad, linker and forward primer (e.g. 314F; 16S) or; R2 = sequence of the combined pad, linker and reverse primer (e.g. 806R; 16S); I = reverse compliment of the R2 primer.

## References


Aponte C, García LV, Marañón T (2013) Tree species effects on nutrient cycling and soil biota: A feedback mechanism favouring species coexistence. For Ecol Manage 309:36-46

Bach LH, Grytnes J-A, Halvorsen R, Ohlson M (2010) Tree influence on soil microbial community structure. Soil Biol Biochem 42:1934-1943

Bahram M, Polme S, Koljalg U, Zarre S, Tedersoo L (2012) Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. New Phytol 193:465-473

Baldrian P (2017) Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol Rev 41:109-130

Bardgett RD, Mommer L, De Vries FT (2014) Going underground: root traits as drivers of ecosystem processes. Trends Ecol Evol 29:692-699

Baumert VL, Vasilyeva NA, Vladimirov AA, Meier IC, Kögel-Knabner I, Mueller CW (2018) Root Exudates Induce Soil Macroaggregation Facilitated by Fungi in Subsoil. Front Environ Sci 6

Bever JD, Platt TG, Morton ER (2012) Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annu Rev Microbiol 66:265-283

Brant JB, Myrold DD, Sulzman EW (2006) Root controls on soil microbial community structure in forest soils. Oecologia 148:650-659

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275-304

Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL (2008) Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. PNAS 105:11505-11511

Bulgarelli D, Garrido-Oter R, Münch Philipp C, Weiman A, Dröge J, Pan Y, McHardy Alice C, Schulze-Lefert P (2015) Structure and Function of the Bacterial Root Microbiota in Wild and Domesticated Barley. Cell Host & Microbe 17:392-403

Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner FO, Amann R, Eickhorst T, Schulze-Lefert P (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488:91-95

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R (2010) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266-267

Chenu C, Sotzky G (2002) Interactions Between Microorganisms and Soil Particles. In: Interactions between soil particles and microorganisms. IUPAC series of Applied Chemistry. pp 3-40.

Coince A, Cordier T, Lengellé J, Defossez E, Vacher C, Robin C, Buée M, Marçais B (2014) Leaf and Root-Associated Fungal Assemblages Do Not Follow Similar Elevational Diversity Patterns. PLOS ONE 9:e100668

Colin Y, Nicolitch O, Van Nostrand JD, Zhou JZ, Turpault MP, Uroz S (2017) Taxonomic and functional shifts in the beech rhizosphere microbiome across a natural soil toposequence. Sci Rep 7:9604

Collins CG, Stajich JE, Weber SE, Pombubpa N, Diez JM (2018) Shrub range expansion alters diversity and distribution of soil fungal communities across an alpine elevation gradient. Mol Ecol

de Freitas CR, Enright NJ (1995) Microclimatic differences between and within canopy gaps in a temperate rainforest. International Journal of Biometeorology 38:188-193

de Graaff MA, Classen AT, Castro HF, Schadt CW (2010) Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytol 188:1055-1064

Dean WE (1974) Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition; comparison with other methods. J Sediment Res 44:242-248

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006a) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069-5072

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006b) Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. Applied and Environmental Microbiology 72:5069-5072

Dray S, Dufour A-B (2007) The ade4 Package: Implementing the Duality Diagram for Ecologists. Journal of Statistical Software 22:1-20

Dukunde A, Schneider D, Schmidt M, Veldkamp E, Daniel R (2019) Tree Species Shape Soil Bacterial Community Structure and Function in Temperate Deciduous Forests. Front Microbiol 10

Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biological Conservation 61:1-10

608 Gale WJ, Cambardella CA, Bailey TB (2000) Root-derived carbon and the formation and stabilization of
609 aggregates. Soil Sci Soc Am J 64:201-207
610 Goberna M, García C, Verdú M (2014a) A role for biotic filtering in driving phylogenetic clustering in
611 soil bacterial communities. Global Ecol Biogeogr 23:1346-1355
612 Goberna M, Navarro-Cano JA, Valiente-Banuet A, García C, Verdú M (2014b) Abiotic stress tolerance
613 and competition-related traits underlie phylogenetic clustering in soil bacterial communities.
614 Ecol Lett 17:1191-1201
615 Gray AN, Spies TA, Easter MJ (2002) Microclimatic and soil moisture responses to gap formation in
616 coastal Douglas-fir forests. Can J For Res 32:332-343
617 Grigulis K, Lavorel S, Krainer U, Legay N, Baxendale C, Dumont M, Kastl E, Arnoldi C, Bardgett RD,
618 Poly F, Pommier T, Schloter M, Tappeiner U, Bahn M, Clément J-C (2013) Relative
619 contributions of plant traits and soil microbial properties to mountain grassland ecosystem
620 services. J Ecol 101:47-57
621 Gweon HS, Oliver A, Taylor J, Booth T, Gibbs M, Read DS, Griffiths RI, Schonrogge K (2015) PIPITS:
622 an automated pipeline for analyses of fungal internal transcribed spacer sequences from the
623 Illumina sequencing platform. Methods Ecol Evol 6:973-980
624 Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk
625 PM, Lücking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ,
626 Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R,
627 Begerow D, Benny GL, Castlebury LA, Crous PW, Dai Y-C, Gams W, Geiser DM, Griffith
628 GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE,
629 Kõljalg U, Kurtzman CP, Larsson K-H, Lichtwardt R, Longcore J, Miądlikowska J, Miller A,
630 Moncalvo J-M, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C,
631 Ryvarden L, Sampaio JP, Schüßler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker
632 C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao Y-J, Zhang N (2007) A higher-level
633 phylogenetic classification of the Fungi. Mycol Res 111:509-547
634 Horner-Devine MC, Bohannan BJM (2006) Phylogenetic clustering and overdispersion in bacterial
635 communities. Ecology 87:S100-S108
636 Hornung M (1985) Acidification of soils by trees and forests. Soil Use Manag 1:24-27
637 Ihrmark K, Bodeker IT, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid J,
638 Brandstrom-Durling M, Clemmensen KE, Lindahl BD (2012) New primers to amplify the fungal
639 ITS2 region--evaluation by 454-sequencing of artificial and natural communities. FEMS
640 Microbiol Ecol 82:666-677
641 IWG W (2007) World reference base for soil resources 2006, first update 2007. FAO, Rome
642 Jarvis SG, Woodward S, Taylor AF (2015) Strong altitudinal partitioning in the distributions of
643 ectomycorrhizal fungi along a short (300 m) elevation gradient. New Phytol 206:1145-1155
644 Jonard M, Nicolas M, Coomes DA, Caignet I, Saenger A, Ponette Q (2017) Forest soils in France are
645 sequestering substantial amounts of carbon. Sci Total Environ 574:616-628
646 Joud D (2006) Guide pour identifier les stations forestières de Rhône-Alpes—Synthèse pour les Alpes du
647 Nord et les montagnes de l'Ain. In: Rhône-Alpes C (ed). p 132.
648 Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO
649 (2010) Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463-1464
650 Kernaghan G, Harper KA (2001) Community structure of ectomycorrhizal fungi across an
651 alpine/subalpine ecotone. Ecography 24:181-188
652 Kohout P, Charvátová M, Štursová M, Mašínová T, Tomšovský M, Baldrian P (2018) Clearcutting alters
653 decomposition processes and initiates complex restructuring of fungal communities in soil and
654 tree roots. ISME J
655 Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M, Bates ST, Bruns TD,
656 Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Duenas M, Grebenc
657 T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lucking R, Martin
658 MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Poldmaa
659 K, Saag L, Saar I, Schussler A, Scott JA, Senes C, Smith ME, Suija A, Taylor DL, Telleria MT,
660 Weiss M, Larsson KH (2013) Towards a unified paradigm for sequence-based identification of
661 fungi. Mol Ecol 22:5271-5277
662 Körner C (2007) The use of 'altitude' in ecological research. Trends Ecol Evol 22:569-574
663 Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index
664 sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq
665 Illumina sequencing platform. Appl Environ Microbiol 79:5112-5120
666 Kuzyakov Y, Blagodatskaya E (2015) Microbial hotspots and hot moments in soil: Concept & review.
667 Soil Biol Biochem 83:184-199

668 Lareen A, Burton F, Schafer P (2016) Plant root-microbe communication in shaping root microbiomes.
669 Plant Mol Biol 90:575-587
670 Le Bissonnais Y (1996) Aggregate stability and assessment of soil crustability and erodibility: I. Theory
671 and methodology. Eur J Soil Sci 47:425-437
672 Lehmann A, Zheng W, Rillig MC (2017) Soil biota contributions to soil aggregation. Nat Ecol Evol
673 Liu J, Ngoc Ha V, Shen Z, Dang P, Zhu H, Zhao F, Zhao Z (2018) Response of the rhizosphere microbial
674 community to fine root and soil parameters following Robinia pseudoacacia L. afforestation.
675 Appl Soil Ecol 132:11-19
676 Lladó S, López-Mondéjar R, Baldrian P (2018) Drivers of microbial community structure in forest soils.
677 Appl Microbiol Biotechnol 102:4331-4338
678 Mao Z, Jourdan C, Bonis M-L, Pailler F, Rey H, Saint-André L, Stokes A (2013) Modelling root
679 demography in heterogeneous mountain forests and applications for slope stability analysis.
680 Plant Soil 363:357-382
681 Mao Z, Saint-André L, Genet M, Mine FX, Jourdan C, Rey H, Courbaud B, Stokes A (2012) Engineering
682 ecological protection against landslides in diverse mountain forests: Choosing cohesion models.
683 Ecol Eng 45:55-69
684 Mao Z, Wang Y, Jourdan C, Cécillon L, Nespoulous J, Rey H, Saint-André L, Stokes A (2015)
685 Characterizing Above- and Belowground Carbon Partitioning in Forest Trees along an
686 Altitudinal Gradient using Area-Based Indicators. Arct Antarct Alp Res 47:59-69
687 Mayfield MM, Levine JM (2010) Opposing effects of competitive exclusion on the phylogenetic structure
688 of communities. Ecol Lett 13:1085-1093
689 McCain CM, Grytnes J-A (2010) Elevational Gradients in Species Richness. In: Encyclopedia of Life
690 Sciences (ELS). John Wiley & Sons, Ltd.
691 McCave IN, Bryant RJ, Cook HF, Coughanowr CA (1986) Evaluation of a laser-diffraction-size analyzer
692 for use with natural sediments. J Sediment Res 56:561-564
693 Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil
694 aggregation. Soil Biology and Biochemistry 22:579-584
695 Miyamoto Y, Nakano T, Hattori M, Nara K (2014) The mid-domain effect in ectomycorrhizal fungi:
696 range overlap along an elevation gradient on Mount Fuji, Japan. Isme j 8:1739-1746
697 Muscolo A, Bagnato S, Sidari M, Mercurio R (2014) A review of the roles of forest canopy gaps. J For
698 Res 25:725-736
699 Muscolo A, Sidari M, Mercurio R (2007) Influence of gap size on organic matter decomposition,
700 microbial biomass and nutrient cycle in Calabrian pine (Pinus laricio, Poiret) stands. For Ecol
701 Manage 242:412-418
702 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB,
703 Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2016) vegan: Community
704 Ecology Package. R package version 2.4-0. https://CRAN.R-project.org/package=vegan.
705 Olsen SR, Cole CV, Watanabe FS, Dean LA, United S, Department of A (1954) Estimation of available
706 phosphorus in soils by extraction with sodium bicarbonate. U.S. Dept. of Agriculture,
707 Washington, D.C.
708 Ostonen I, Truu M, Helmisaari HS, Lukac M, Borken W, Vanguelova E, Godbold DL, Lohmus K, Zang
709 U, Tedersoo L, Preem JK, Rosenvald K, Aosaar J, Armolaitis K, Frey J, Kabral N, Kukumagi M,
710 Leppalammi-Kujansuu J, Lindroos AJ, Merila P, Napa U, Nojd P, Parts K, Uri V, Varik M, Truu
711 J (2017) Adaptive root foraging strategies along a boreal-temperate forest gradient. New Phytol
712 215:977-991
713 Parks DH, Beiko RG (2013) Measures of phylogenetic differentiation provide robust and complementary
714 insights into microbial communities. ISME J 7:173-183
715 Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the
716 microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789-799
717 Poirier V, Roumet C, Angers DA, Munson AD (2018) Species and root traits impact macroaggregation in
718 the rhizospheric soil of a Mediterranean common garden experiment. Plant Soil 424:289-302
719 Prescott CE, Grayston SJ (2013) Tree species influence on microbial communities in litter and soil:
720 Current knowledge and research needs. For Ecol Manage 309:19-27
721 Price MN, Dehal PS, Arkin AP (2010) FastTree 2 – Approximately Maximum-Likelihood Trees for
722 Large Alignments. PLOS ONE 5:e9490
723 Prieto I, Roumet C, Cardinael R, Dupraz C, Jourdan C, Kim JH, Maeght JL, Mao Z, Pierret A, Portillo N,
724 Roupsard O, Thammahacksa C, Stokes A, Cahill J (2015) Root functional parameters along a
725 land‐use gradient: evidence of a community‐level economics spectrum. J Ecol 103:361-373

Ren C, Zhang W, Zhong Z, Han X, Yang G, Feng Y, Ren G (2018) Differential responses of soil microbial biomass, diversity, and compositions to altitudinal gradients depend on plant and soil characteristics. Sci Total Environ 610-611:750-758
Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584
RStudio Team (2016) RStudio: Integrated Development for R. RStudio Inc., Boston, MA URL
Saetre P, Bååth E (2000) Spatial variation and patterns of soil microbial community structure in a mixed spruce–birch stand. Soil Biol Biochem 32:909-917
Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. ISME J 6:1749-1762
Shea F, Watts CE (1939) Dumas method for organic nitrogen. Industrial & Engineering Chemistry Analytical Edition 11:333-334
Shen C, Liang W, Shi Y, Lin X, Zhang H, Wu X, Xie G, Chain P, Grogan P, Chu H (2014) Contrasting elevational diversity patterns between eukaryotic soil microbes and plants. Ecology 95:3190-3202
Shi S, O'Callaghan M, Jones EE, Richardson AE, Walter C, Stewart A, Condron L (2012) Investigation of organic anions in tree root exudates and rhizosphere microbial communities using in situ and destructive sampling techniques. Plant Soil 359:149-163
Siles JA, Margesin R (2016) Abundance and Diversity of Bacterial, Archaeal, and Fungal Communities Along an Altitudinal Gradient in Alpine Forest Soils: What Are the Driving Factors? Microb Ecol 72:207-220
Singh D, Takahashi K, Kim M, Chun J, Adams JM (2012) A hump-backed trend in bacterial diversity with elevation on Mount Fuji, Japan. Microb Ecol 63:429-437
Tindall BJ, Rossello-Mora R, Busse HJ, Ludwig W, Kampfer P (2010) Notes on the characterization of prokaryote strains for taxonomic purposes. International journal of systematic and evolutionary microbiology 60:249-266
Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. J Soil Sci 33:141-163
Urbanová M, Šnajdr J, Baldrian P (2015) Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biol Biochem 84:53-64
Uren NC (2000) Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In: The rhizosphere. CRC Press, pp 35-56.
Uroz S, Buée M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2:281-288
Uroz S, Oger P, Tisserand E, Cébron A, Turpault MP, Buée M, De Boer W, Leveau JHJ, Frey-Klett P (2016) Specific impacts of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities. Sci Rep 6:27756
van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, Van de Voorde TFJ, Wardle DA (2013) Plant–soil feedbacks: the past, the present and future challenges. J Ecol 101:265-276
Van Soest PJ (1963) Use of detergents in the analysis of fibrous feeds. 2. A rapid method for the determination of fiber and lignin. Journal of the Association of Official Agricultural Chemists 46:829-835
Wang J, Soininen J, He J, Shen J (2012) Phylogenetic clustering increases with elevation for microbes. Environ Microbiol Rep 4:217-226
Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology 73:5261-5267
Wang Y, Kim JH, Mao Z, Ramel M, Pailler F, Perez J, Rey H, Tron S, Jourdan C, Stokes A (2018a) Tree root dynamics in montane and sub-alpine mixed forest patches. Ann Bot 122:861-872
Wang Y, Mao Z, Bakker MR, Kim JH, Brancheriau L, Buatois B, Leclerc R, Selli L, Rey H, Jourdan C, Stokes A (2018b) Linking conifer root growth and production to soil temperature and carbon supply in temperate forests. Plant Soil 426:33-50
Webb CO (2000) Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. The American Naturalist 156:145-155
Yang B, Pang X, Hu B, Bao W, Tian G (2017a) Does thinning-induced gap size result in altered soil microbial community in pine plantation in eastern Tibetan Plateau? Ecology and evolution 7:2986-2993

785 Yang Y, Geng Y, Zhou H, Zhao G, Wang L (2017b) Effects of gaps in the forest canopy on soil microbial
786 communities and enzyme activity in a Chinese pine forest. Pedobiologia 61:51-60
787 Zhang J, Kobert K, Flouri T, Stamatakis A (2014) PEAR: a fast and accurate Illumina Paired-End reAd
788 mergeR. Bioinformatics 30:614-620
789 Zhang Q, Goberna M, Liu Y, Cui M, Yang H, Sun Q, Insam H, Zhou J (2018) Competition and habitat
790 filtering jointly explain phylogenetic structure of soil bacterial communities across elevational
791 gradients. Environ Microbiol 20:2386-2396

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